Book of Abstracts

International Conference on Antimicrobial Research

Valladolid, Spain. 3-5 November 2010

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ICAR2010

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TOPICS

ICAR will provide a new forum in Europe for the presentation, exchange and dissemination of information and experiences on anti-microbe strategies (against bacteria, fungi or protozoans), in biotic or abiotic environments, in planktonic or adhered states, in biologically specific or unspecific ways, *in vitro* or *in vivo*, in a general context marked by the threat posed by the increasing antimicrobial resistance of pathogenic microorganisms. "Anti" is here taken in a wide sense as "against cell cycle, adhesion, or communication", when harmful for human health, industry or economy (infectious diseases, chemotherapy, food, biomedicine, agriculture, livestock, biotechnology, water systems...). It will include topics on antimicrobial resistance, (early) microbial and resistance detection, enhancement of innate defences against pathogens, as well as methods & techniques. Topics will include:

Antimicrobial chemistry (experimental and computational). Analytical detection of antibiotics in complex samples.
Synthesis and screening of novel chemical compounds for antimicrobial action. Natural, synthetic and semi-synthetic antibiotics. Analogs. Structural determination. *In-silico/ab-initio/de-novo* antimicrobials discovery. New targets for antimicrobials. Rational design of antimicrobials. Bioinformatics and comparative genomics for the identification of antimicrobial targets…

Antimicrobial natural products.
Antimicrobial substances from terrestrial and marine organisms. Antimicrobial peptides. Antimicrobial enzymes. Essential oils. Bioactive phytochemicals. Plant/Herbal extracts. Purification. Structural determination…

Antimicrobials mechanisms of action.
Methods and Techniques.

Antimicrobial resistance. Superbugs. Multi-resistant strains. Emerging and re-emerging pathogens.
Microbial resistance to antibiotics and biocides. Molecular mechanisms. Resistance genes. Prevention of resistance. Surveillance & statistics. Genetics and Proteomics. Emerging and re-emerging bacteria and fungi in humans, animals, and plants. Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin Intermediate/Resistant *Staphylococcus aureus* (VISA/VRSA), *Clostridium difficile*, *Mycobacterium tuberculosis*, Vancomycin-resistant *enterococcus* (VRE), *Cryptosporidium*, *Plasmodium parasite*, *Plasmodium falciparum*, *Leishmania* species, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, *Cryptococcus*, *Escherichia coli* O157:H7, *Helicobacter* spp., *Enterobacter sakazakii*, *Serratia* spp., Fluoroquinolone-Resistant *Pseudomonas aeruginosa* (FQRP)...

Antimicrobial microbes.
Microbial-derived toxins. Bacteriocins (colicins, microcins, lantibiotics…). Archaeocins. Biocontrol approach to microbial invasions (probiotics, lactic acid bacteria…). Biosynthesis of antibiotics. Genetic and metabolic engineering. Gene regulation…

Antimicrobial viruses.
Bacteriophages. Phage therapy and biocontrol in humans, animals (agriculture-farm animals, aquaculture), plants, food industry… Materials functionalization with bacteriophages. Using bacteriophages for microbiological detection…

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Antimicrobial, anti-adhesive surfaces & coatings. Microbial adhesion to surfaces. Biofouling. Biofilm formation, control and eradication. Novel characterization techniques. Physical and chemical (inorganic (e.g. silver, copper compounds) and organic) surface modification. Cationic surfaces. Functionalization strategies for polymers, metals, metal oxides, ceramics. Drug-eluting concepts. Biofilms susceptibility to antimicrobials. Antibiotic resistance of microorganisms in biofilms. Genomics and Proteomics...

Antimicrobials in consumer products.
Textiles (hygienic clothing, activewear, medical textiles…), paper industry, active packaging (food industry…), public buildings (hospitals, schools, restaurants, day care centers, nursing homes…). Safety and toxicological aspects…

Antimicrobial physics.
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Non-antibiotic biocides. Hygiene and Sterilizing.
Disinfectants, antiseptics, preservatives… Mechanism of action. Resistance to non-antibiotic biocides. Combination of physical and chemical treatments. Hygiene and Sterilizing. Sanitizers. Regulatory issues. Good practices…

Techniques and Methods.
Susceptibility Testing. Rapid microbial and resistance detection. Detection of antibiotics in environmental samples. Microscopy, microanalysis & spectroscopy, single-cell studies, high-throughout studies, nanomechanical studies, microfluidics, lab-on-a-chip concepts, miniaturized science, analysis of microbial surfaces, heterogeneity, statistics. Interaction of antimicrobial drugs with model membranes. Analytical techniques…

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Interfering microbe-microbe communication (quorum sensing) as antimicrobial strategy.

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Antimicrobials evaluation. Pre-clinical and clinical trials.

Public awareness, learning & teaching, influence on policy-makers. Regional regulatory frameworks and experiences on antimicrobials.

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1. Natural Products I: Antimicrobial Peptides

A new class of lipidic immunomodulators that activate TLR-dependent cascades and cytokine secretion.

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Cationic lipids are positively charged amphiphilic molecules which, for most of them, form positively charged liposomes, sometimes in combination with a neutral helper lipid. Such liposomes are mainly used as efficient DNA, RNA or protein carriers for gene therapy or immunization trials. Over the past decade, significant progress has been made in the understanding of the cellular pathways and mechanisms involved in lipoplex-mediated gene transfection (1) but the interaction of cationic lipids with cell components and the consequences of such an interaction on cell physiology remains poorly described. Recently, trying to explain the immunoadjuvant properties (2) of a cationic lipid discovered in our group (N-t-butyl-N'-tetradecyl-3-tetradecylamino-propionamidine-diC14-amidine), we identified its agonistic interaction with the Toll-like receptor 4 (TLR4), the natural sensor of LPS (the bacterial lipopolysaccharide). This activation seems specific of the shape and characteristics of the molecule since increasing the length of the hydrocarbon chains by 2 methyl groups suppresses its activity. This cationic lipid has only limited features in common with LPS species from different bacterial origin such as short hydrocarbon chains and the presence of a polar headgroup. However, the number of chains (2 versus generally 6 to 7) and the polar headgroup (small alkylated cationic amidinium group versus the bulky polar anionic headgroup of LPS) make the two molecules look very different but surprisingly such dissimilar structures are capable of activating the same receptor(3). As compared with other adjuvants being currently developed, the chemistry of amidine derivatives is rather simple, allowing the quick development and screening of new derivatives.

1-Elouahabi A, Ruysschaert JM.- Mol Ther. 2005 -11(3):336-47
2- Jacquet A, Vanderschrick JF, Vandenbranden M, Elouahabi A, Magi M, Garcia L, RuysschaertJM Mol Ther. (2005) 11, 960-968
3-Lonez C, Vandenbranden M, Ruysschaert JM.- Prog Lipid Res. 2008 Sep-47(5):340-7

A new class of Scots pine antimicrobial proteins, which act by binding β-glucan

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In the Northern Hemisphere, conifers are a highly abundant and economically important plant group. One of the most destructive plant pathogens, *Heterobasidion annosum*, causes root and butt rot disease in conifers. Scots pine, Norway spruce and Douglas fir serve as the main hosts for this pathogen. The fungi degrade both lignin and cellulose components of wood, which contributes to their action as strong parasites and saprophytes, destroying living roots and stems of all ages, as well as dead trees.

Like other plants, conifers respond to pathogen attacks by producing pathogenic related (PR) proteins, many of which have been shown to have antimicrobial activity, and so are also referred to as antimicrobial peptides/proteins (AMPs). In particular, Scots pine (*Pinus sylvestris*) secretes a number of small, closely related disulfide-rich proteins in response to challenges with fungal pathogens like *Heterobasidion annosum*, although their function is presently unknown. The proteins, which we call Sp-AMP1-5, were originally identified as highly up-regulated genes in infected roots, representing a new family of plant proteins. In this study we have examined the expression patterns, structure and function of these proteins. Expression patterns were observed using Northern blots and quantitative real time PCR. One of the genes (Sp-AMP3) was cloned, and the protein was expressed in *Pichia pastoris*. The protein was purified, then biologically and biochemically tested, including binding assays and microscopic analysis of the protein's effects on fungi. Homology modelling and sequence comparisons were used to investigate its structure.

The Sp-AMP3 proteins were shown to be up-regulated after treatment with salicylic acid and an ethylene precursor, 1-aminocyclopropane-1-carboxylic-acid, but neither methyl jasmonate nor hydrogen peroxide induced their expression. The pure Sp-AMP3 samples clearly possessed antifungal activity against *H. annosum*, in both vegetative growth and spore germination assays (Fig.1). Microscopic observations verified the morphological changes caused by the Sp-AMP3 in fungal hyphae and spores. Furthermore, Sp-AMP3 was shown to bind to soluble and insoluble β-1,3-glucans with high affinity. Homology modelling and sequence comparisons indicated that all five Sp-AMPs would exhibit a Greek-key β-barrel structure. A likely binding site was identified as a conserved patch on the protein surface that can accommodate approximately four sugar units.

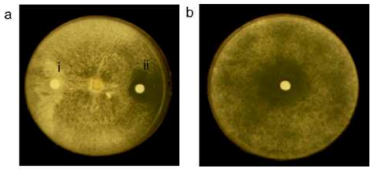

Figure 1: Inhibition of (a) vegetative growth, and germination by Sp-AMP.

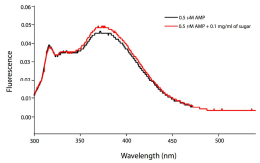

Figure 2: Fluorescence changes of SP-AMP3 in the (b) spore presence of laminaribiose

We conclude that the SpAMP proteins represent a new class of antimicrobial proteins that act by binding β-glucans, a major component of fungal cell walls.

Keywords: antimicrobial proteins, β -1,3 glucan, binding, Heterobasidion annosum, homology modelling, inhibition, pathogen, Pinus sylvestris.

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Antimicrobial Activity of Human Beta Defensin-2 and Human Beta Defensin-3 against Methicillin Resistance *Staphylococcus aureus* (MRSA) and Sensitive *Staphylococcus aureus*.

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Human beta-defensins (HBDs) are an intrinsic part of the innate immune response. These peptides have been shown to possess varying potencies against a wide spectrum of bacteria, viruses and fungi. Due to increasing bacterial resistance to traditional antimicrobials, increasing interest is being shown in these peptides as possible alternative therapeutic molecules.

In this study, the bactericidal activity of two peptides (HBD-2, and HBD3) was assessed against a range of *Staphylococcus aureus* (*S. aureus*) strains, including MRSA, using a standard bactericidal assay. Briefly, 10^5 bacterial cells were incubated with the test peptide (0.03, 0.3 or 3 ug/mL) in a low salt buffer for 30 min at 37 ^{o}C, after which viable counts were taken.

The antibiotic sensitive strains (*S. aureus* NCTC 10655, NCTC 8331, NCTC 8511, NCTC 10970, and NCTC 9717) were more susceptible to killing and exhibited a dose response to the test peptides reaching 99% kill at the highest test concentration. The MRSA strains (MRSA 100, 99, 98, 4, and 7all clinical isolates from NHS London) although still susceptible to the peptides showed less percentage kill at all test concentrations, reaching a maximum at 68% kill. Interestingly, for the first time we report a clear and significant difference in bactericidal activity of defensins towards Methicillin sensitive and Methicillin resistant strains of *S. aureus* (p=0.0005). Studies aimed at identifying the mechanism for such a difference could throw light on the killing action of defensins and have implications for their use as therapeutic molecules.

Keywords; Human Beta Defensins, MRSA, HBD-2, HBD-3, Bactericidal activity.

Antimicrobial aza-β^3-peptides : Structure-activity relationship ?

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Designing antimicrobial molecules based on pseudopeptides to increase the activity, selectivity and bioavailability of natural peptides is now widespread. Recently, numerous peptidomimetics have been developed for biological applications including azapeptides, β-peptides, peptoids, oligoureas In this context, aza-β^3-aminoacids were used as new blocks to compose antimicrobial peptide sequences. These monomers are analogs of β^3-aminoacids in which the CH_α is replaced by a nitrogen stereocenter conferring a better flexibility to the pseudopeptide due to the side chain beared on a chiral nitrogen atom with a non-fixed configuration. The nonnatural oligomers have an extended conformational space and are supposed to adopt non-canonical secondary structures.

From a natural antimicrobial peptide, depending on the aza-β^3 residue insertions, the modifications can result either in inactive pseudopeptides or in a drastic enhancement of the antimicrobial activity without cytotoxicity. To understand how the incorporation of aza-β^3-aa modulates the antimicrobial activity, we propose to study the structure of aza-β^3-peptides by CD and NMR spectroscopy. To date, no solution structures of peptides containing aza-β^3-aa have been solved. Crystalline structures of linear aza-β^3-peptides and aza-β^3-cyclopeptides have been determined and exhibit an internal hydrogen-bond network leading to bifidic eight-membered ring pseudocycles, called N-N turn or hydrazino turn. Recently, it was demonstrated that the nitrogen configuration inversion could be fixed, by incorporating chiral monomers among these heteromacrocycles.

We have determined the first three-dimensional structures of naturally related linear pseudopeptides containing aza-β^3-aa in SDS micelles. Insertions of aza-β^3-aa systematically break the natural antimicrobial peptide amphipatic helices and lead either on stable hydrazinoturn conformation or in flexible unordered structures. When one modified residues is incorporated in a linear sequence, the hydrazinoturn seems to be classically surrounded by β-turns stabilizing the overall peptide structure over 5 residues. This typical fold was not observable if several aza-β^3 residues are inserted in closer positions destabilizing this motif. We studied the propagation of the hydrazino/β-turns succession on model peptides with sequences based on aza-β^3 residues all the 3 residues: αα(aza-β^3)αα(aza-β^3)αα…Structure and activities will be presented depending on the modifications..

Keywords pseudopeptides; structure-activity relationships

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Antimicrobial Responses in the Male Reproductive Tract of Lipopolysaccharide Challenged Rats.

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Innate immune machinery including the Toll-like receptors (TLRs) confers the first line of defense mechanisms to counter pathogenic microorganisms that enter the body. The male reproductive tract is vulnerable to infection and the role of TLRs and the antimicrobial responses that operate to counter infections in this organ system are poorly understood. In this study we provide the first line of evidence that the male reproductive tract induces the expression of antimicrobial genes namely, *Spag11* variants and defensins when challenged with lipopolysaccharide (LPS) with a concomitant increase in protein expression. However, there was an inverse relationship between induction of antimicrobial gene expression and plasma testosterone. An increase in the mRNA levels of proinflammatory cytokines was observed parallel to the induction of *Spag11* variants and majority of defensin expression in the male reproductive tract. The increase in *Spag11* and defensin mRNA in response to endotoxin administration demonstrates their importance in protecting the male reproductive tract during infection. Results of this study help to understand male reproductive tract innate immune defense mechanisms and to design novel peptide antibiotics to prevent sexually transmitted diseases.

Keywords: Antimicrobial, defensin, epididymis, toll-like receptor.

Antimicrobial activity, gastrointestinal protection and immunological role of milk goat SAA3 (M-SAA3) and derivative peptides

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Serum Amyloid A3 (SAA3) is an acute phase protein mainly produced extrahepatically. Although its role is not clearly understood, functions related to protection of the organism through milk ingest and activation of the immunological system have been described (McDonald et al., 2001, Molenaar et al., 2009)). The objective of this study was the evaluation of the antibacterial activity and gastrointestinal protection and immunological role of milk-derived goat SAA3 (M-SAA3) and related peptides. Goat M-SAA3 was recombinantly obtained. Total RNA was isolated from milk and retrotranscribed to cDNA. Specific SAA3 PCR was performed and the product was cloned into a pET101 vector, sequenced and expressed in *Escherichia coli* BL21 (DE3). M-SAA3 was purified by affinity chromatography using a 6 histidine tail which was introduced in position N and C-terminal of the protein. Sequence analysis allowed the design of 10-mer derived peptides. Total and partial aminoacid scrambled peptides were used as controls. Human SAA3 derived peptide was used as specie specific control and *Lactobacillus rhamnosus* as a probiotic control in gastrointestinal protection in vitro studies. Human intestinal Caco-2 cell line was incubated with 100 µg/ml of peptides, PBS or 10^8 cfu/well of *L. rhamnosus* for 1h. A total of 10^6 cfu/well of enteropathogenic *E. coli* (EPEC) was added and incubated for 2h. Cells were washed and EPEC was released and quantified by viable cell count in McConkey plates. Pro-inflammatory interleukin-8 (IL-8) expression was measured in the gastrointestinal cell line by real-time PCR. For antimicrobial assays $5 \cdot 10^5$ cfu/ml of EPEC or *Staphylococcus aureus* were incubated with 25, 50 or 100 µg/ml of peptides, recombinant protein or PBS in 96-well plates at 37ºC. Bacterial growth was determined by optical density and bacterial viability was evaluated by viable cell count in McConkey or Nutrient Broth plates. EPEC binding to Caco-2 cells diminished 3 and 2 orders of magnitude after incubation with goat or human SAA3-derived peptides respectively. Also, it was demonstrated 99% of attenuation of IL-8 expression in the human intestinal cell line after incubation with the goat-derived M-SAA3 peptide. In addition, M-SAA3 derived peptides described a direct antimicrobial effect against EPEC and *S. aureus*, with a difference of 2 and 1 order of magnitude in their respective viability. The recombinant goat M-SAA3 decreased cell viability of *S. aureus* up to 50% but had no effect in EPEC. In conclusion, goat M-SAA3 and its related peptides seem to have protective and immunological roles against gastrointestinal infections in vitro and an antimicrobial function under the conditions described herein.

Keywords: Serum amyloid A3, peptides

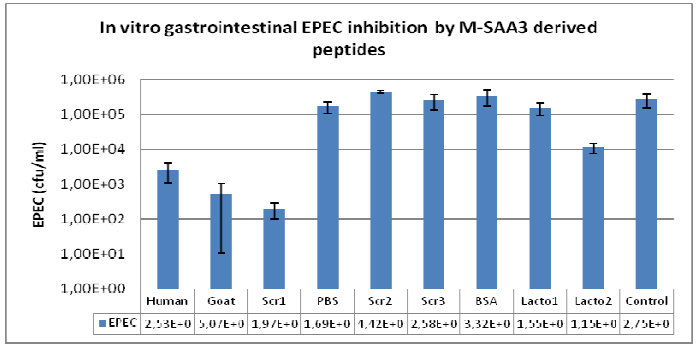

Figure1.- EPEC viability (cfu/ml) after infection of human Caco-2 cell line in the presence or not of different M-SAA3 derived 10- mer peptides. Human: human-derived peptide. Goat: goat-derived peptide. Scr1: partially scrambled goat-derived peptide. Scr2: total scrambled bovine-derived peptide. Scr3: total scrambled goat-derived peptide. BSA: 10-mer N-terminal BSA derived peptide. Lacto 1 and Lac2: 10^8 and 10^9 cfu/ml of *L. rhamnosus*. PBS: PBS incubation. Control: no additive or buffer added.

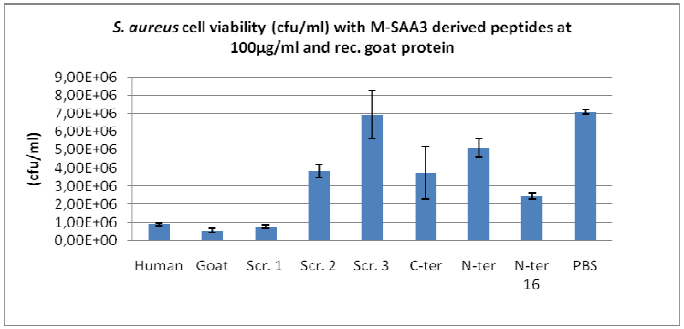

Fig. 2. *S. aureus* cell viability (cfu/ml) after incubation with 100µg/ml of M-SAA3 derived peptide (human, goat or Scr1, Scr2, Scr3) or goat M-SAA3 recombinant protein (C-ter N-ter: rec. goat M-SAA3 with histidine tail at C- or N-terminal position N-ter16: rec. goat M-SAA3 obtained at16ºC expression or PBS as negative controls).

Apoptotic volume decrease induced by lactoferrin in *Candida albicans* cells

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Human lactoferrin induces an apoptosis-like phenotype in *Candida albicans* cells, which includes phosphatidylserine exposure, nuclear chromatin condensation, DNA degradation, and increased reactive oxygen species (ROS) production(1). The candidacidal activity of lactoferrin appears to be a apoptotic mechanism involving the K^+-channel mediated K^+-efflux, an event similar to that observed in the apoptotic process of higher-eukaryotic cells(1,2). Lactoferrin caused a loss of cell viability concomitant with a decrease in cellular volume. Yeast membrane transporters have a well-defined role in cell volume regulation, and yeast cells decrease their volume by efflux of Cl^- and K^+ ions, with concomitant movement of water out of the cells. We observed a volume decrease following lactoferrin treatment of cells suggesting that lactoferrin may activate transport of anions parallel to efflux of K^+ and water from *C. albicans*. The protection against lactoferrin-induced volume reduction provided by DIDS, an inhibitor of volume-regulating Cl^--channels, and observed in our assays may be a result of DIDS inhibition of outward Cl^- efflux preventing cell volume loss. Our data suggest that a key determinant in the apoptotic mechanism of lactoferrin in *C. albicans* cells is disruption of the regulatory circuits for ion transport and cell volume homeostasis.

Furthermore, the mechanisms associated to the reported(2) inhibition of NaCl on the candidacidal activity of lactoferrin were studied and will be discussed.

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Keywords: lactoferrin, apoptosis, *Candida albicans*

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Boosting of Host Innate Immunity for Protection Against Infectious Diseases

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Innate immune system, conserved through evolution across plants, insects, and humans, is the first line of host defense against the invading pathogens. The abilities of the host to first recognize and then to clear the pathogen are the two essential steps of the innate immune defense. This allows rapid elimination of the pathogen before it can cause inflammation or disease in the host. However, many pathogens have developed resistance either by blocking the recognition or the clearance step of the host innate immune processes. One striking example of such pathogens is the gram-negative bacterium *Xylella fastidiosa (Xf)*. So far different Xf strains have been identified to cause infection in 70 different plant hosts. The most economically devastating diseases are: Pierce's Disease in grapes and Variegated Chlorosis in citrus. In all diseases, Xf is transmitted by sharpshooter vectors to the plant xylem, which is deficient in nutrients and plant immune defense. It appears that Xf tends to colonize in the xylem and thereby blocks the flow of nutrients and water from the roots. This leads to inflammation and scorching of the leaves. By extensive analysis of multiple Xf genomes, we have identified the outer membrane protein B (mopB) as a putative therapeutic target that remains invariant across all strains. We have also demonstrated that mopB is the most abundant protein on the Xf membrane and is critical for pathogen growth, viability, and colonization. By searching through the host innate immune repertoire, we are able to identify and show that an elastase can cleave the recombinant mopB. However, many of the elastase sites of mopB are hidden in the periplasm of a live Xf. In order to access both extracellular and periplasmic sites on mopB, we have fused the elastase with cecropin using a flexible linker. The idea was that the lipophilic and lytic cecropin would be able to penetrate the outer-membrane, carry the elastase to the periplasm to complete the cleavage of mopB, and through the synergy of cleavage and lytic functions the chimera would facilitate rapid Xf clearance. Indeed, we have been able to generate a transgenic grape line that produces the elastase-linker-cecropin chimera in the plant xylem (the very site of infection), clears the pathogen, and completely protects against infection. This is the first example in which a chimeric protein is expressed locally at the site of infection in a transgenic plant to protect against the disease.

This has encouraged us to extend the therapeutic approach using a protein chimera into a general strategy for overcoming pathogenic resistance against host innate immune defense. The strategy involves the design of a protein chimera in which the functional domains for pathogen recognition and clearance are linked together in the same protein. The protein chimera simultaneously targets the two conserved sites of the pathogen thereby limiting the possibility of pathogenic resistance through concomitant mutations at the two sites. The synergy of recognition and clearance ensures rapid killing of the pathogen. This approach has recently been successful for *in vitro* protection against infection by *Staphylococcal aureus* (a human pathogen). The possibility of converting our strategy into a universal therapy against viral, bacterial, and fungal pathogens seems logical and appealing.

Branched Peptides with High Charge Density and Enhanced Anti-Microbial Properties

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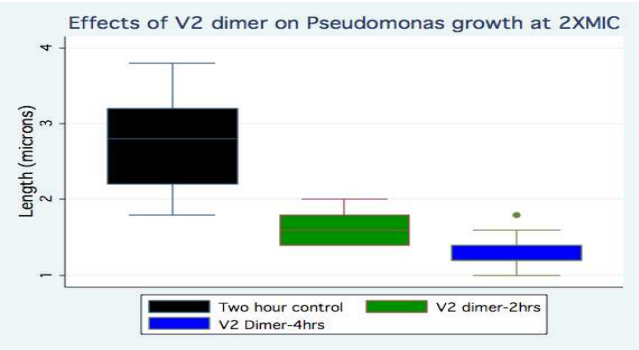
Small branched peptides were found to exhibit potent broad spectrum antimicrobial activity against a panel of pathogens including clinically challenging multidrug-resistant *P. aeruginosa*, methicillin-resistant *S. aureus*, and fungi and the properties were superior to a monomer of the same group, as well as to a standard ophthalmic antibiotic (gentamicin). Electron microscopy and other studies showed rapid bactericidal action (Fig 1). Increasing the number of branch points significantly increased the antimicrobial activity by several fold compared to the linear monomer. The branched peptides had virtually no host cell toxicity even at 200 μg/ml. In a laboratory simulation of antibiotic resistance, the V2-D did not develop resistance to gentamicin-resistant *P. aeruginosa*. NMR studies of the linear monomer and V2-D were determined in DPC:POPC mixed micelles showed that linear monomer formed a non-covalent dimer whereas V2-D existed as a monomer. The surface plots and the isoelectrostatic potential maps suggested the presence of localized positive electrostatic potential close to the branch point and removal of an additional negative charge in V2-D which was not observed in the linear monomer. **Conclusion:** The findings support the role of charge density of designed peptides with high selectivity for killing pathogens. The excellent antimicrobial properties, minimal host cell toxicity and inability of bacteria to form resistance highlights the importance of a branched design as important for the development of a new class of anti-infective agents.

Keywords: anti-microbial, branched peptide, charge density

Fig 1. Results from scanning electron microscopic studies of the growth of Pseudomonas (ATTC standard) at 2, 4 hrs after exposure to the V2-D peptide at 2 X the MIC_{99} value.



Clong and sequence ananlysis of cDNA enconding the antibacterial peptide, from the mosquito Anopheles sinensis

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Objective To clone cDNA encoding the antibacterial peptide , defensinA from mosquito *A nopheles sinensis* and conduct sequence analysis. **Methods** Total RNA was extracted from *A n. sinensis* , primers def1 and def2 were synthesized according to literary , RNA sequence of the adult of *A n. sinensis* was amplified by RT-PCR to get the defensin cDNA which was then cloned into T vector and sequenced. The cDNA sequence of defensin encoding gene was analysed and compared with other defensin gene of insect . **Results** It was found that a DNA f ragment encoding defensin about 308 bp in length was obstained from *A n. sinensis* cDNA. It s 308bp nucleotide sequences and deduced 64 amino sequences shared highly identity with *A n. gambiae* defensin encoding sequence(95 %) . It was evaluated *A n. sinensis* defensin encoding gene is a new gene. It was given a name is *A n. sinensis* defensisA. **Conclusion** *A n. sinensis* defensinA encoding gene have been successfully cloned. These will provide for a basis to study the gene.

Key words: *A nopheles sinensis* ; antibacterial peptide ; defensinA ; cDNA clone ; sequence analysis

First 3D NMR structures of chicken defensin and hen egg defensin : Insights into the structure-activity relationships

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First 3D structure of a chicken defensin

Salmonella is a bacterial enteropathogen representing the main cause of food poisoning worldwide. Persistence of Salmonella Enteritidis in poultry flocks in the form of intestinal carriage represents an important source of contamination of poultry derived food products. Improving immunity of the mucosal barrier of the bird intestine to fight bacterial colonisation is a strategic alternative to the controversial use of antibiotics. During the study of chicken response to Salmonella infection, it had been shown that avian β-defensin genes (AvBD1 and 2) were highly expressed in intestinal tissue of birds resistant to Salmonella colonisation.

In the present study, the NMR structure of synthetic chicken AvBD2 was performed to progress in the understanding of structure-function relationships for avian defensins by comparison with the only bird defensin 3D structure actually known : the structure of king penguin AvBD103b, previously called spheniscin, we determined a few years ago[1]. Chicken AvBD2 presents the typical 3-stranded β-sheet structure of β-defensins but lacks the N-terminal helix observed for example for human defensins. Remarkably AvBD2 and AVBD103b do not present any amphipathic surface usually required for the interaction of antimicrobial peptides with the bacterial membrane. Antimicrobial activities have been determined for these two molecules, in a wide range of Gram- and Gram+ bacteria[2,3]. In the light of these two 3D structures, we analyzed the consensus sequence of avian β-defensins, in order to get new insights in the structural and/or functional involvement of specific residues in the antimicrobial activity. Establishing such relationships is essential to understand avian β-defensin mechanism of action, and is consequently essential to propose strategic modifications of β-defensins, that may improve potency and/or selectivity against antibiotic-resistant bacteria. We thereby highlighted well-conserved residues which could have a role in the biological function. The first synthesized mutant shows a significantly decrease of the activity and structural modifications (to be submitted).

First 3D structure of a hen egg defensin

The chicken egg is a model of particular interest since it contains all the components that are essential for embryonic development and protection in a closed chamber exposed to a putatively aggressive milieu. Among the first antimicrobial proteins identified in eggs, galline was chosen for chemical synthesis and structural analysis. This is the first study of the 3D NMR structure of a hen egg defensin: from the disulfide bridges arrays observed, it can be classified in the β-defensin family. We present here the structural comparison of galline with AvBD103b and AvBD2 bird defensins. Moreover, on the base of this first 3D structure for an egg defensin, we built 3D structures of other egg proteins by homology modelling, to draw the first features specific to egg (to be submitted).

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Keywords : 3D Structure; NMR; structure-activity; modelling; defensin; bird, egg,

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Frog skin antimicrobial peptides promote survival of *Caenorhabditis elegans* infected by a multi-drug resistant strain of *Pseudomonas aeruginosa*

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In the past two decades, numerous families of genetically encoded antimicrobial peptides (AMPs), from all living organisms, have been described [1,2]. They are conserved components of the innate immune response, and represent the first host defence line against microbial infections. Alghough AMPs show a marked variation in size, sequence and structure, most of them are polycationic and fold into an amphipathic helical or beta-sheet structure, a feature which aids their interaction and insertion into microbial membranes that are believed to be the principal target for their killing mechanism. Before reaching the negatively-charged bacterial membrane, AMPs need to bind, via electrostatic interactions, the anionic components of the microbial cell surface and diffuse through the cell wall. The growing emergence of multidrug-resistant (MDR) microorganisms makes it increasingly difficult to treat infections. These infections include those associated with *Pseudomonas aeruginosa*, which is hard to eradicate, especially in patients with a compromised immune system[3]. Amphibian skin is one of the richest sources for such AMPs, but only a few studies on their *in vivo* activity and mode of action have been reported. Here we investigated: (i) the activity and mechanism underlying the killing of short AMPs from frog skin (e.g., temporins and esculentin fragments[4]) on a MDR clinical isolate of *P. aeruginosa*; (ii) their *in vivo* antimicrobial activity and mode of action, using the mini-host model of *Caenorhabditis elegans*[5]. Our data revealed that *in vivo*, both temporin-1Tb and esculentin(1-18) were highly active in promoting the survival of pseudomonas-infected nematodes, although temporin-1Tb did not show significant activity *in vitro,* under the experimental conditions used. Importantly, esculentin(1-18) permeated the membrane of *Pseudomonas* cells within the gut of the infected nematode. To the best of our knowledge, this is the first report showing the ability of an AMP to permeate the microbial membrane within a living organism. Besides shedding light on a plausible mode of action *in vivo* of frog skin AMPs, our data suggest these peptides as templates for the design and development of new anti-infective agents.

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Keywords antimicrobial peptides; frog skin; antibiotic resistance.

Identification of K^+-channel involved in the apoptotic death of *Candida albicans* cells induced by lactoferrin

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Human lactoferrin induces an apoptosis-like phenotype in *Candida albicans* cells, which includes phosphatidylserine exposure, nuclear chromatin condensation, DNA degradation, and increased reactive oxygen species (ROS) production(1). We have previously reported the critical role of K^+-channel mediated K^+-efflux in the yeast *C. albicans*, an event similar to that observed in the apoptotic process of higher-eukaryotic cells(1,2). The K^+-efflux was an essential event to initiate the apoptotic machinary as suggested the inhibition caused by several inhibitors of K^+-channels. However, cells treated with the K^+-ionophore valinomycin to deplete intracellular potasium were unable to initiate an apoptosis-like process, and only a delayed colony-growth was observed. The possible causes of this different mode of action of valinomycin and lactoferrin will be discussed. The identification of the K^+-channel involved in the K^+-efflux induced by lactoferrin was performed using a pannel of *C. albicans* strains with different mutations (*TRK1*/*trk1*; *TOK1*/*tok1*; *tok1*/*tok1*) two known K^+-channels of this species, Trk1p and Tok1p.

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Keywords: lactoferrin, K^+-channel, *Candida albicans*

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Inhibition of the blood stages of the human malaria parasite, *Plasmodium falciparum*, by membrane-active cyclic peptides

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Malaria is endemic in 107 third world countries, with an estimated 400-500 million clinical malaria cases per year. The development of antimalarial drugs is essential to curb the spread of the resistant forms of human malaria parasites, in particular resistant *Plasmodium falciparum*. During its residence inside the erythrocyte, the *P. falciparum* parasite extensively customises the host cell membrane to meet its requirements. We hypothesised that the modification of the host erythrocyte membrane by the infecting parasite may render it vulnerable to selective recognition and perturbation by membrane-active compounds, such as antimicrobial peptides. Our studies focussed on small stable cyclic peptides with antimicrobial and membrane activity. We showed that gramicidin S, a cyclic decapeptide, almost relied solely on the selective lysis of the erythrocyte However, the analogous tyrocidines, a group of membrane active cyclic decapeptide antibiotics form *Bacillus aneurinolyticus* selectively inhibit malaria parasite blood stages, possibly by a non-lytic mechanism of action focussed on the tropozoite developmental stage. The antiplamodium activity and selectivity of the tyrocidines was highly dependent on the tyrocidines primary structure and QSAR data pointed to potential pharmacophore structure and character for selective activity. Antiplasmodial activity was also observed for selected members in a group of antimicrobial model peptides that share some characteristics with the tyrocidines. These peptides also consists of short conformationally constrained sequences that are rich in particular amino acid residues, such as charged (arginine, lysine, ornithine) and aromatic (tryptophan, phenylalanine, tyrosine) residues. Although the most active cyclic hexapeptides only inhibited parasites in the micromolar range, possibly via a membrane associated mechanism, they were highly selective for the infected erythrocytes. Micromolar activity was also observed in a small library of retrocyclins, cyclic octadecapeptides, derived from the anti-HIV Θ-defensins. The retrocyclins are highly selective with virtually no cytotoxicity and specifically inhibited the reinfection of erythrocytes by the merozoite stage. It was also shown that the retrocyclins inhibit HIV entry into a variety of cells. The coincidental anti-malaria activity of these anti-HIV peptides makes them an interesting group to pursue.

To conclude, the small and stable cyclic peptides with antiplasmodial activity are a promising group for development as antimalarial drugs, especially for use in severe resistant malaria and as last resort drugs. Also, the inherent membranolytic mode of action of antimicrobial peptides with anti-plasmodium activity, as well as the possibility that there are intraparasitic targets, limits the possibility of acquired parasite resistance, the biggest problem for sustainable control of malaria..

Keywords malaria; tyrocidines, cyclic peptides

Interaction Mechanisms Between Mycobacteria and Antimicrobial Peptides

Max Koistinen, Sam Willcocks, Arne Böhling, Gesa Helms, Ulrich Schaible and Thomas Gutsmann

Tuberculosis kills 2 million people per year worldwide. Mycobacterium tuberculosis is resistant against a large number of host defence peptides, which are active against other Gram-negative or –positive bacteria. One reason for this resistance is the unique structure of the mycobacterial cell wall. Mycobacteria produce a thick mycolate-rich outer covering which functions as an exceptionally efficient barrier.

In this work we focused on the membrane forming properties of trehalose dimycolate (TDM) as one of the important components of the mycobacterial outer barrier. We reconstituted pure TDM layers and lipid mono- and bilayers consisting in addition of PE:PG as model matrix. We investigated the properties of these layers by means of film balance measurements, atomic force microscopy (AFM), and reconstituted planar lipid bilayers. TDM alone formed very stable monolayers and, when embedded into the lipid matrix, self-aggregated into stable domains. Using force spectroscopy we observed that TDM layers had higher mechanical stability as compared to pure phospholipid membranes. Furthermore, reconstituted TDM-containing membranes could not be permeabilized by the antimicrobial peptide LL32, which is the highly active fragment of human cathelicidin. In killing experiments, we could show that LL32 is not active against *M. bovis* BCG from which TDM was purified. In contrast, a derivative of the defensin hBD-3 can permeabilize the membrane and kill the mycobacteria with high efficiency. We propose that TDM contributes to the stability of the mycobacterial cell wall and strongly impairs the membrane permeabilization by host defence peptides.

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Interaction of antimicrobial peptides with OmpF containing LPS membranes

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More and more bacteria are resistant against classical antibiotics. For this reason antimicrobial peptides are used to treat bacterial infections.

To get a better understanding how antimicrobial peptides (AMP) interact with the outer membrane of Gram-negative bacteria this work is focused on the interaction between antimicrobial peptides and OmpF containing LPS membranes. It is known that AMPs interact with this outer membrane but they also interact with proteins. The outer membrane of Gram-negative bacteria is asymmetric and contains lipopolysaccharides on the outer and phospholipids on the inner leaflet. A number of proteins, for example the outer membrane protein F (OmpF), are located in the membrane. OmpF is a water filled pore and small molecules (<600Da) like ions, sugar molecules, or antibiotics can pass it. Furthermore, our experiments show that also peptides can interact with the porins leading to a change in the porin function.

The experiments were performed using asymmetric Montal-Mueller membranes resembling the outer membrane. They were composed of LPS from *E.coli* strain WBB01 and a mixture of phospholipids (Phosphgatidylethanolamine: Phosphatidylglycerol: Diphosphatidylglycerol, 81:17:2). The activity of four different AMPs (LL20, hBD-3-l, Poly-L-lysine (PLL) and Polymyxin B (PMB)) on the membrane and porin function was characterized. Only hBD-3-l and PLL were directly interacting with the OmpF leading to a closing of the porin molecules. In contrast PMB and LL20 led to an increased intercalation of the porins into the planar lipid bilayers. To confirm the results obtained from experiments using the reconstituted membranes microbiological experiments were performed. Furthermore, biophysical techniques including FRET-experiments were used to characterize the molecular interactions.

In summary we could show that there is a strong interaction between the outer membrane of Gram-negative bacteria, their porin OmpF, AMPs, and classical antibiontic (e.g. enrofloxacin).

Investigation of the effect of cationic antimicrobial peptides on bacteria

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Despite the use of antibiotics for nearly eight decades, infectious diseases continue to have an impact both on human morbidity and mortality worldwide. A consequence of anti-infective agent use is the increasing prevalence of resistance, which subsequently places a major burden on healthcare services. The search for new antibacterials is becoming increasingly urgent, focussing on agents that are active against a wide range of bacteria, possess minimal host toxicity and do not readily select for resistant mutants. Previously under-developed areas of research such as cationic antimicrobial peptides (CAPs) are being re-investigated and infrequently used antibacterials are being re-assessed.

Colistin, (polymixin E) was introduced into clinical practice approximately 60 years ago but was rarely used clinically between 1980-2000, due to the reported high rate of nephrotoxicity associated with this compound. However a recent study has shown that the total cumulative dose of colistin is associated with nephrotoxicity, not the daily dose.

CAPs are gene encoded host defence peptides which are chemically diverse and effective antibacterials. Constitutively expressed or induced, endogenous CAPs are found in tissues of most organisms. As part of the innate immune system CAPs provide a rapid response to bacterial invasion, are quickly up-regulated after microbial infection and rapidly neutralise a broad range of microbes.

The aim of this research was focussed on examining the effects of colistin and novel CAPs on *Escherischia coli* NCTC 4174. Results obtained via flame photometry, scanning electron microscopy and flow cytometry will be presented, illustrating the effect of these cationic antibacterials on this bacterium.

From these results it will be evident that the bactericidal effect of these polypeptides is not solely due to membrane disruption. Incubation with 0.5mg/ml (2xMIC) of NP108 caused 30% potassium loss in *E. coli* within approximately 5 minutes. Incubation with 30µg/ml colistin merely induced <5% potassium loss over 24 hours. SEM analysis revealed aggregation in *E. coli* post colistin exposure at sub and supra-inhibitory concentrations (Figure 1). Several changes were observed in bacteria incubated with NP101 and NP108 corresponding to modes of action previously attributed to peptides. Cellular aggregation, gross deformation of bacterial cells and inhibition of septation are some of the effects observed. Data from flow cytometry revealed the inability of *E. coli* to withstand either colistin at 3µg/ml (MIC), NP108 at 0.25mg/ml (MIC) or 0.5mg/ml (MIC) NP101.

Experimental data suggests modes of action other than membrane disruption may be responsible for the bactericidal action of colistin, or the CAPs NP101 and NP108.

Keywords; bacterial resistance, novel cationic antimicrobial peptides, colistin

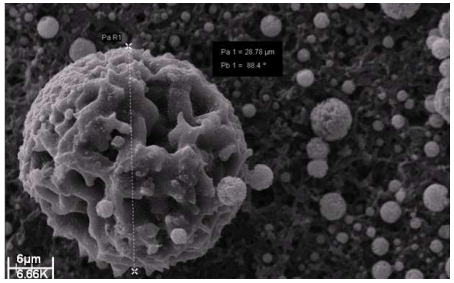

Figure 1:*E. coli* incubated with 3µg/ml (MIC) of colistin for 24 hours

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Isolation of a new antimicrobial/antitumor plant peptide: Biotechnological prospects for its use in cancer and infectious diseases therapies

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The immune system of multi-cellular organisms comprises a vast arsenal of mechanisms to protect the host from the constant interactions with infectious microorganisms. Antimicrobial peptides (AMPs) are peptides which protect their hosts against a vast array of microorganisms. These peptides are produced by several species including bacteria, insects, plants, vertebrates and they have been recognized as remotely evolved molecules that have been effectively preserved in mammals. AMPs are expressed on the primary barriers of the organism such as skin and mucosal epithelia, preventing the colonization of host tissues by pathogens. We have previously reported the induction after infection and the cytotoxic activity of potato aspartic proteases (*St*APs) towards plant pathogens. Here we show results on the antimicrobial/antitumor activities of these enzymes and a domain from these enzymes, named *St*Asp-PSI. *St*Asp-PSI has structural homology with a family of proteins with antimicrobial/antitumor activity, the SAPLIPs family. The results obtained show that *St*Asp-PSI is able to kill spores of two potato pathogens but not plant cells, in a dose-dependent manner. As reported for *St*APs (*Solanum tuberosum* aspartic proteases), *St*Asp-PSI ability to kill microbial pathogens is dependent on the direct interaction of the protein with the microbial cell wall/or membrane, leading to increased permeability and lysis. Additionally, we demonstrated that, like proteins of SAPLIP family, *St*Asp-PSI and *St*APs are cytotoxic for Gram negative and Gram positive bacteria in a dose-dependent manner. The amino acid residues conserved in SP_B (pulmonary surfactant protein B) and *St*Asp-PSI could explain the cytotoxic activity exerted by *St*Asp-PSI and *St*APs against Gram positive bacteria.

On the other hand, results obtained show that *St*APs induce apoptosis on Jurkat T cells at short time of incubation in a dose dependent manner. However, not significative effect on the T lymphocytes viability was observed at all times and *St*APs amounts assayed. *St*Asp-PSI was able to induce DNA fragmentation; ROS generation and cell cytotoxicity on human breast cancer cells in a dose-dependent manner. These results open a new perspective to analyze these proteins as possible candidates to develop new drugs that would be active against microbes but not against mammalian cells and consider these proteins as conceptually promising agents in cancer therapy.

Keywords: plant-specific insert, antimicrobial peptides, SAPLIP family.

Isolation of antimicrobial peptides from plant *Tetragonia tetragonioides*

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Resistant microorganisms are becoming a great problem for public health. Beside classical antibiotics there are existing natural resistance mechanisms based on excretion of other substances. In our research we are trying to find those substances responsible for rapid antimicrobial mechanism with lower potential to cause resistance. Such substance seems to be antimicrobial peptides [1], part of innate immunity, synthesized by all living organisms. In our project leaves of *Tetragonia tetragonioides* were chosen as a source of these peptides.

Leaves of *Tetragonia tetragonioides* were homogenised and extracted to Tris/HCl buffer with the addition of proteases inhibitors. After removing of solid particles the supernatant was gradually precipitated by ammonium sulphate to remove large proteins and the supernatant was dialysed. The membrane filtration was used as a next separation step, to gain peptide fractions under 30 kDa, and SPE column with C18 filling to resolve them into hydrophilic and hydrophobic part. These parts were performed by RP-HPLC and obtained fractions were tested for antimicrobial activity and characterized by mass spectrometry analysis.

Antimicrobial activity of fractions was screened by diffusion method on agar plates. Tested organisms were gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, gram-positive bacteria: *Staphylococcus aureus, Micrococcus luteus*, *Listeria innocua* and fungi: *Candida scotii, Aspergillus ochraceus* and *Mucor species*.

After RP-HPLC we got 28 fractions isolated from hydrophobic part and 8 fractions from hydrophilic part. Two of the hydrophilic fractions and eight of the hydrophobic ones showed significant inhibition of bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The mass spectrometry analysis confirmed presence of low molecular peptides at 6 active fraction (M_W 112 – 4 894 Da) without detailed identification. In the future there is planned N-terminal sequence analysis of these antimicrobially active peptides.

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Keywords antimicrobial peptides; isolation, *Tetragonia tetragonioides*

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Isolation of peptides with antimicrobial activity using diverse purification steps and its sequences determination

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Microbiological research is focused on solution of pathogen resistance problem. The number of resistant pathogenic microorganisms has increased in last years. In the future there will be fewer possibilities to cure multiresistance infections by existing antibiotics. One of the potential solutions seems to be isolation or design of the short cationic peptides naturally synthetized as a part of innate immunity[1]. In our project larvae of the fleshly *Neobellieria bullata* were chosen as a source of these peptides.

The haemolymph was picked up from larvae, gradually centrifuged and precipitated by acidified methanol. Subsequently these fractions were separated by chromatographic methods (SPE column, FPLC, RP-HPLC) to obtain fractions of short peptides. Identification and characterization of these fractions were performed by tricine electrophoresis, mass spectrometry MALDI-TOF analysis and N-terminal sequencing.

Antimicrobial activity of fractions was screened by diffusion method. Testing model organisms were gram-negative bacteria: *Escherichia coli, Pseudomonas aeruginosa*, gram-positive bacteria: *Staphylococcus aureus, Bacillus megaterium, Listeria innocua*, and fungi: *Candida scotii, Aspergillus ochraceus* and *Mucor species.*

Several fractions showed antimicrobial activity against bacteria *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus megaterium* and fungi *Candida scotii*. Tricine electrophoresis proved presence of low molecular peptide. MS analysis of antimicrobial active fractions determined molecular masses less than 10 kDa and the N-terminal sequencing gave us several sequences but not completed. The sequences do have very low similarity with peptides in the NCBI databases or with the database of antimicrobial peptides [2] and seem to be very unique.

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Keywords antimicrobial peptides; isolation, N-terminal sequencing, *Neobellieria bullata*

Lactoferrin inhibits DNA-Induced IFN-α by Human Plasmacytoid Dendritic Cells

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Bacterial as well as self DNA is released at sites of infection due to cell lysis. This DNA induces the production of IFN-α by $TLR9^+$ Plasmacytoid dendritic cells (pDCs), which in turn can lead to exacerbated tissue damage as seen in psoriasis and lupus. Antimicrobial peptides like cathelicidins enhance the production of IFN-α, but little is known about the effect of other antimicrobial proteins stored in neutrophils. Here we present the inhibitory capacity of neutrophil-derived lactoferrin in activating DNA-induced IFN-α production by human plasmacytoid dendritic cells. Confocal microscopy images show lactoferrin binding to DNA molecules or CpG oligonucleotides outside the cell, preventing their uptake by pDCs, and the subsequent activation of TLR-9. Flow cytometry experiments with labeled CpGs to analyze internalization dynamics indicate that lactoferrin prevents cathelicidin-DNA complexes from being internalized, avoiding over-activation of TLR-9. Moreover, when lactoferrin was supplemented on diet, mice orally treated with the irritant DSS presented lower IFN-α levels on the mesenteric lymph nodes than the controls. Therefore, we propose that lactoferrin acts as a scavenger molecule for DNA motifs, potentially reducing the undesirable effects of high levels of IFN-α.

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Making the Knot: Structure-Activity Relationships of Lasso Peptides

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Mechanically-interlocked molecular architectures, such as molecular knots, arise from topological arrangements instead of traditional bonds. Lasso peptides are ribosomally assembled peptides produced by bacteria that share such an unusual knotted structure, where an 8 to 9 residues macrolactam ring closed between the Gly1/Cys1 N-terminus and the carboxyl side chain of an Asp/Glu at position 8/9 is penetrated by the C-terminal tail that is threaded and sterically maintained inside the ring. Lasso peptides have diverse properties, including antibacterial activities, as exemplified by microcin J25 (MccJ25)[1], capistruin[2] or lariatins[3]. Although their modes of action are diverse, lasso peptides generally bind proteins, acting as enzyme inhibitors or receptor antagonists. As such, the MccJ25 antibacterial activity has been shown to involve recognition step by a siderophore receptor and subsequent inhibition of the target RNA polymerase, two processes to which the ring and the tail regions contribute separately.

In current study we have generated several MccJ25 analogues by site-directed mutagenesis, varying the amino acid composition and the size of the ring or the tail region. As the lasso fold is a prerequisite for bioactivity, we addressed the question whether these variants can still adopt their original knotted structures by a combination of physicochemical and biochemical methods (mass spectrometry, NMR, circular dichroism and digestion by proteases). The variants' structures were correlated to their antimicrobial activities. The results presented here pinpoint the regions that are susceptible to modifications while not affecting the lasso structure, paving the way for targeted peptide engineering.

Keywords lasso peptide; antimicrobial peptide; structure-activity relationships; mass spectrometry; NMR

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Non-lytic Penetration of Protamine, an Arginine-rich Cationic Antimicrobial Peptide Into Gram-negative Bacteria

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Cationic antimicrobial peptides (CAPs) are usually amphipathic scaffolds that allow interaction with, and damage to Gram-negative envelopes while others such as the arginine-rich peptides, kill bacteria without any obvious lytic effects but through interactions with intracellular targets. Some of the non-lytic peptides partially share common sequence homologies that are sometimes arginine-rich (Ar-CAPs).

Ptm, a unique 4200 Da arginine-rich CAP, naturally occurring in fish milt, has been shown in the past to rapidly lyse susceptible Gram positive and Gram negative targets. Unlike most other CAPs, Ptm is not amphipathic and lacks secondary structure due to the even distribution of positive charges along the peptide backbone. Ptm from herring milt (clupeine) has 20 arginine residues and is the most highly cationic naturally-occurring Ar-CAP reported to date with a pI of 11-13. During the last two decades since their discovery, the question of how Ar-CAPs translocate across the bacterial outer membrane has remained unanswered.

We have determined that Ptm internalizes *E. coli, P. aeruginosa* and *S. typhimurium* without permanent poration or lysis although it is apparently not translocated across symmetrical reconstituted phospholipid bilayer membranes. Furthermore, we have shown with computer modeling studies that the probability of Ptm crossing a symmetrical phospholipid bilayer membrane is very low.

In order to test the hypothesis that Ptm internalization requires the presence of certain membrane (porin) proteins, we inserted purified cation-specific porins OmpF, OmpD and OprF isolated from *E. coli* 29522, *S. typhimurium* 14028 and *P. aeruginosa* PAO1 27853, respectively, and found evidence of Ptm translocation reflected by fluctuations in the transmembrane ion current, reflecting the molecular interactions with the channel wall. The study was complemented with computer based molecular modeling and immunoelectron microscopy and we suggest here for the first time that Ptm may be internalized via porins.

Keywords: *Protamine, Porins, OmpF, OmpD, OprF*

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Peptidoglycan hydrolase antimicrobials for staphylococcal pathogens that are refractory to resistance development

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Staphylococci are both human and agricultural pathogens that are demonstrating an increasing frequency of antibiotic resistance. The rise in bacterial resistance to antibiotics world-wide has precipitated the search for alternatives to broad range antimicrobials, and specifically those that are refractory to resistance development. Peptidoglycan hydrolases (including bacteriophage endolysins) are one such group of novel antimicrobials. These enzymes cause Gram positive cell lysis by degrading cell wall peptidoglycan, are often genus specific, and due to co-evolution it is believed rare that a bacterial host can evade its phage endolysin. Lysins are modular in structure, often with multiple lytic domains and a cell wall binding domain. We have shown that fusing the active domains from multiple endolysins generates chimeric molecules that maintain their parental specificities and lytic activities. It is rare that a bacterium can resist three simultaneous lytic activities, thus we predict that these fusions will be highly refractory to resistance development, and in vitro data supports this hypothesis. Here we describe fusion antimicrobials that each lyse staphylococci with three unique and synergistic lytic activities. The fusion proteins are examined in bactericidal assays against multiple pathogens and for efficacy in blood and in animal infection models. Efforts to target intracellular pathogens with fusion peptidoglycan hydrolases will also be discussed.

Keywords: peptidoglycan hydrolase, *Staphylococcus aureus*, LysK, Lysostaphin, electron spray ionization mass spectrometry

Sensitization of *Pseudomonas aeruginosa* to antibiotics by human lactoferricin derived peptides persists several hours after peptide removal

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Pseudomonas aeruginosa is a gram-negative pathogen causing serious nosocomial infections in immunocompromised patients. *P. aeruginosa* is very resistant to antibiotics, and this fact greatly reduces the therapeutic options. Previous studies have shown that certain cationic peptides derived from human lactoferricin: i) permeabilized the cell envelope of *P. aeruginosa* and enhance the action of antibiotics at concentrations below their minimum inhibitory concentration (MIC); ii), are rapidly lethal at concentrations above their MIC. However, it is not known if these compounds have postantibiotic effect (PAE), a parameter of high therapeutic interest that quantifies the capacity of an antimicrobial drug to inhibit the growth of bacteria after removal of the drug.

Using a *P. aeruginosa* clinical strain highly resistant to antibiotics, we demonstrated that treatment with one of the peptides (P4-9) at twice its MIC causes a 0.83 hours delay in *P. aeruginosa* growth. In addition, we found that pre-treatment with P4-9 produces a cell damage that persists for several hours after drug removal. During this interval, bacteria are sensitive to antibiotics at sub-inhibitory doses indicating a persistent bacterial cell permeabilization. Moreover, using a *P. aeruginosa* mutant overexpressing the efflux pump MexAB-OprM system, we found that pre-treatment with P4-9 causes a long term sensitization of the mutant to antibiotics that are known substrates of the system. We have designated this effect as Post-antibiotic Permeabilizing Effect (PAPE).

Keywords: cationic peptide, postantibiotic effect, *P. aeruginosa,* permeabilization.

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Structural and functional diversity of natural antimicrobial oligopeptides

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Chemical substances named as oligopeptides consist of 2 to 50 amino acid residues [1]. To date, primary structures of more than 10000 endogenous oligopeptides have been identified. Natural oligopeptides may regulate nearly all vital processes. Few integrated biological processes are known which are not regulated, or at least modulated, by small peptides. Such roles are especially well known in the regulatory organ systems, viz. nervous, endocrine and immune systems, but their functions extend well beyond the bounds of single organ systems or even of single biological species. Natural antimicrobial oligopeptides have been discovered in practically all living organisms. Whereas in prokaryotes these oligopeptides regulate competition between separate species occupying ecological niches and function as the signaling molecules in processes of intercellular communication, in eukaryotes they are localized in every organ and tissue and play a key role in innate immunity.

This study was performed using our EROP-Moscow (**E**ndogenous **R**egulatory **O**ligo**P**eptides) database (http://erop.inbi.ras.ru/) [2]. It contains complete information on natural oligopeptides and demonstrates that chemical structures of natural oligopeptides have been identified from more than 2000 different species representing all the biological kingdoms. More than 2100 oligopeptides possess antimicrobial functions. These substances were found out in animals (1699), plants (322), bacteria (130), fungi (42), and viruses (2). It is known 132 human oligopeptide structures. Primary structures of antimicrobial oligopeptides are characterized as having widely diverse sequences. Many of them display a net positive charge, ranging from +2 to +18. Nearly all antimicrobial oligopeptides form amphipathic structures upon interaction with target membranes and exhibit antimicrobial activity against a wide variety of micro-organisms. Antimicrobial oligopeptide structure and function offers hope for discovery and development of improved agents to prevent or treat infectious diseases caused by pathogens that resist conventional antimicrobial agents. Various human oligopeptides show a potent effect on pathogenic micro-organisms including antibiotic-resistant bacteria. Moreover, human defensins indicate that these oligopeptides are involved in various biological processes associated primarily with defensive and regulatory responses to infections by pathological agents.

The functional class of antimicrobial oligopeptides involves structures, which display an unusually broad spectrum of antibacterial, antifungal, antiviral, and antitumor activities. These peptides are able to suppress or kill not only Gram-negative and Gram-positive bacteria, but also fungi, parasites, cancer cells, as well as HIV virus and herpes simplex virus. Antimicrobial oligopeptides that are known today, produced in response to infection or injury, are quite selective for microbes over eukaryotic cells. In animals, antimicrobial peptides are found in different body parts and organs most likely to come into contact with pathogenic microbes. They have been detected in the skin, ears, and eyes, on epithelial surfaces of the tongue, tracheas, lungs, and gut, and in the bone marrow and testes; in blood they are most prevalent in neutrophils.

Interest in antimicrobial, especially antibacterial oligopeptides is determined by their medicinal potential. This is particularly important because of the continuous emergence of novel strains of bacteria that are resistant to natural and synthetic antibiotics, which prompts the search for new efficacious remedies against pathogenic bacteria. The functional properties of antibacterial oligopeptides are realized in different ways depending on the peculiarity of their structure. These peptides are characterized by compact, completely or partially helical structures formed with cysteine bridges as well as by disordered structures. An important role in formation of disordered oligopeptide structures belongs to proline residues, which constitute a large part of the total number of amino acid residues in relatively short oligopeptide molecules [3].

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Keywords: antimicrobial; oligopeptide; EROP-Moscow database

Synergistic effects of acylated Ghrelin on antimicrobial activities mediated by LL-37

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Background: Ghrelin (GHR) was originally found as an appetite hormone peptide secreted from endocrine cells of the stomach and pancreas to stimulate hunger. The active form of GHR has an n-octanyl modification at serine3-residue in a total 28-amino-acid peptide (acylated-GHR), and this modification is essential for its interaction with the receptor in brain because its non-modified form (des-acyl-GHR) lacks hormonal activity. Interestingly, recent studies revealed that GHR is present in saliva as well as blood. Based on the similarity of cationic property between GHR and antimicrobial peptide LL-37, as well as the ectopic secretion of GHR in saliva, we have hypothesized that GHR may possess antimicrobial activity in the context of oral-gastrointestinal mucosa.

Objectives: To examine 1) if GHR possesses antimicrobial activity and 2) if GHR can alter the antimicrobial activity mediated by LL-37.

Methods: Bactericidal activities of acylated-GHR and des-acyl-GHR were examined against three different strains of microorganisms: *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. In addition, synergistic or additive effects of acylated-GHR and des-acyl-GHR on the antimicrobial activities of LL-37 were evaluated in the presence or absence of a physiological concentration of NaCl (150 mM).

Results: GHR demonstrated bactericidal activities against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Acylated-GHR, but not des-acyl-GHR, also displayed synergistic effects on LL-37-mediated bactericidal activities against all three bacteria tested. Furthermore, the synergistic effect of acylated-GHR on LL-37-mediated bactericidal activity was maintained in the presence of a physiological concentration of NaCl (150 mM), while bactericidal effect of LL-37 or acylated-GHR, respectively, was attenuated remarkably in the presence of NaCl (150 mM). In contrast, des-acyl-GHR showed no synergistic or additive effects on LL-37-mediated bactericidal activities.

Conclusion: The results indicated that GHR may function as an antimicrobial peptide in the physiological context of oral-gastrointestinal mucosa, especially mediating strong antibacterial synergism with LL-37. (Supports: NIH grant DE-018310 from the National Institute of Dental and Craniofacial Research and Pilot Grant from Harvard Catalyst)

Keywords : Ghrelin, LL-37, antimicrobial activity

The antifungal protein AFP from Aspergillus giganteus inhibits the viability of *Saccharomyces cerevisiae* strains deficient in cell wall integrity

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The antifungal protein AFP, secreted by *Aspergillus giganteus* is able to inhibit the growth of a variety of filamentous fungi (e.g. *A. fumigatus*, *A. niger*, *Fusarium oxysporum*) by causing membrane invaginations and membrane permeabilisations. However, the protein does not negatively affect the growth of yeast, bacteria or mammalian cell types. Our recent work demonstrated that AFP inhibits chitin synthase activities in sensitive fungi which is counteracted by increased activities of the cell wall integrity pathway (CWIP).

In order to understand the resistance mechanism of yeast strains, we have screened *S. cerevisiae* mutants deleted for the components of the CWIP and chitin synthesis. Most of the ~ 70 screened strains remained AFP-resistant, except the knock out mutants' Δwsc1, Δtor1, Δvps34 and Δchs1, which became moderate sensitive towards AFP. The plasma membranes of these mutants became readily permeabilized by AFP. Interestingly, the presence of AFP provoked increased chitin synthesis in these strains, an observation which we also made for the AFP-resistant filamentous fungus *Penicillium chrysogenum* and the moderate sensitive mutant of *F. oxysporum* ΔchsV.

The obtained results stipulate the hypothesis that moderate-sensitive and resistant filamentous fungi counteract AFP inhibitory effects by strongly increasing their chitin levels, thereby making the cell walls presumably more rigid. However, this response does not occur in AFP-sensitive fungi. Apparently, the classical CWIP is not sufficient to counteract AFP inhibitory effects and seems not to be involved in increased chitin synthesis. Our findings give rise to the assumption that fungal strains which only use the classical CWIP are AFP-sensitive.

Keywords: Antifungal protein, Cell wall integrity pathway, mode of action AFP, *Aspergillus giganteus*

The antifungal protein AFP from *Aspergillus giganteus* prevents *Fusarium* growth of barley during the malting process

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Contamination and spoilage of crop and biomaterials by filamentous fungi is responsible for enormous economical losses worldwide. Globally, head blight caused by *Fusarium* species is mainly responsible for crop losses. However, not only crop loss is a thread but also mycotoxin formation and fungus specific metabolites cause serious food safety and quality problems. Especially, secondary growth of *Fusarium* species during malting is a great concern in beer production and has been made responsible for the so called gushing effect of bottled beer.

The filamentous fungus *Aspergillus giganteus* produces a selectively acting antifungal protein, named AFP. This protein acts fungicidal at micromolar concentration against a wide range of plant-pathogenic fungi, whereby growth and viability of bacteria, yeasts, plant- or human cells remains unaffected. It has been demonstrated that especially *Fusarium* species are very sensitive towards AFP.

We have thus tested the applicability of AFP during the malting process using barley samples naturally infested with *Fusarium* species. Our results indicate that AFP applied during the malting process is indeed able to inhibit the growth of *Fusarium* species, whereby the malt quality is not negatively affected. Current investigations are focusing on the evaluation of the impact of AFP on the end product beer; the detection of *Fusarium* metabolites and the analysis of the gushing potential.

Keywords: Antifungal protein AFP, Barley, Malting, Fusarium, Mycotoxin, Bio-control

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The antimicrobial target of lactoferrin is the bacterial translocating-proton ATPase

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Two bacterial species, *Pseudomonas aeruginosa* and *Lactococcus lactis*, with different metabolic features, were used as a comparative experimental model to investigate the antimicrobial target and mechanism of transferrins. In anaerobiosis, *P. aeruginosa* cells were not susceptible to lactoferrin (hLf) or transferrin (hTf). In aerobiosis, the cells were susceptible but O_2-consumption was not modified, indicating that components of the electron transport chain (ETC) were not targeted. However, the respiratory chain inhibitor piericidin A significantly reduced the killing activity of both proteins. Moreover, 2,6-dichlorophenolindophenol (DCIP), a reducing agent that accepts electrons from the ETC coupled to H^+-extrusion, made *P. aeruginosa* susceptible to hLf and hTf in anaerobiosis. These results indicated that an active cooperation of the cell was indispensable for the antimicrobial effect. In *L. lactis* cells lacking an ETC, the absence of a detectable transmembrane electrical potential in hLf-treated cells suggested a loss of the H^+-ATPase activity. Furthermore, the inhibition of ATPase activity and H^+-translocation (inverted membrane vesicles) provided direct evidence of the ability of hLf to inhibit H^+-ATPase in *L. lactis*. Based on these data, we propose that hLf and hTf also inhibit the H^+-ATPase of respiring *P. aeruginosa* cells. Such inhibition thereby interferes with re-entry of H^+ from the periplasmic space to the cytoplasm, resulting in perturbation of intracellular pH and the transmembrane proton gradient. Consistent with this hypothesis, periplasmic H^+ accumulation was prevented by anaerobiosis or by piericidin A, or induced by DCIP in anaerobiosis. Collectively, these results indicate that transferrins target H^+-ATPase and interfere with H^+-translocation, yielding a lethal effect *in vitro*.

Keywords: lactoferrin, transferrin, H^+-ATPase, antimicrobial mechanism

The role of gram-negative envelope LPS on the bactericidal properties of proteins and peptides: The case of Eosinophil Cationic Protein.

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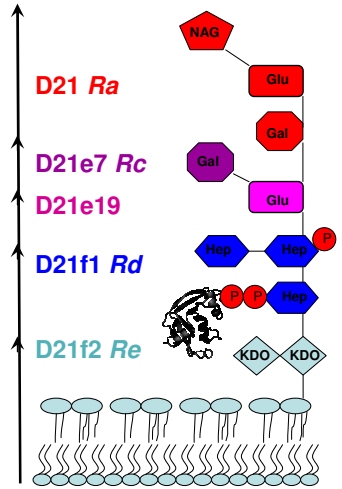
We are working on the human Eosinophil Cationic Protein (ECP) as model of antimicrobial protein involved in host defense. ECP is highly cationic and its bactericidal activity is dependant on its membrane destabilization activity[1]. Moreover, the protein presents a high affinity to lipopolysaccharides (LPS) and can specifically agglutinate gram –negative bacteria cells[2]. We have identified a region at the N-terminus, ECP(1-45), that can reproduce most of the protein activity, and partly retain the cell aggregation properties[3].



To better understand ECP antimicrobial mechanism of action and in order to specifically evaluate the contribution of the LPS components on the protein antimicrobial properties, we have assayed a battery of *E. coli* K12 strain variants which present distinct chemotypes at the LPS core structure (Figure). The selected *E. coli* K12 mutants are defective in some of the genes involved in the LPS synthesis and present progressively truncated LPS molecules at their outer membrane. The so called "rough" bacteria mutants (*Ra* to *Re* chemotypes) provide a useful system to assess *in situ* the particular role of oligosaccharide and anionic LPS substituents.

We have measured the ECP effect at both the outer envelope and cytoplasmic membrane level and assessed the changes on the bacteria cell viability. The bacteria cell agglutination activity and the outer envelope damage was further inspected by Scanning Electron Microscopy. Additionally, the protein bactericidal performance has been compared to the N-terminus peptide ECP(1-45). The data correlates the LPS structure with the bacteria viability and reveals the key contribution of electrostatic interactions between the cationic polypeptide and the anionic groups at the LPS inner core. Therefore, the results corroborate that both the length and charge of the LPS alters the bacteria cell susceptibility to the protein cytotoxicity.

Keywords Lipolysaccharides, Eosinophils, gram–negative bacteria

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Two centrocin-like peptides from the green sea urchin, *Strongylocentrotus droebachiensis*

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Antimicrobial peptides (AMPs) play a significant role in the innate immune system in both vertebrates and invertebrates and might have promising properties for drug development or for other purposes.

Today, two families of AMPs, named strongylcins and centrocins, have been isolated and characterized from the green sea urchin, *Strongylocentrotus droebachiensis.* Strongylocins are cysteine rich with a mature peptide size of 5.6-5.8 kDa. Centrocins are heterodimer structured peptides, 4.5-4.5 kDa, containing a heavy chain and a light chain, which is linked by a single cysteine disulfide bond. The genome of the closely related species, the purple sea urchin, *S. purpuratus*, was shown to contain two putative proteins with high similarity to the centrocins. In addition, two recombinant strongylocins from *S. purpuratus* showed activity against Gram-negative and Gram-positive bacteria.

At the meeting, we will present data of two centrocin-like AMPs purified from coelomocyte extracts of this sea urchin. These native peptides are cationic and show potent activities against Gram-positive and Gram-negative bacteria. The peptides have an intramolecular heterodimeric structure. The pharmacophore is believed to be situated at the heavy chain.

Keywords sea urchin, echinoderm extracts, antimicrobial peptides, innate immunity, marine bioprospecting, intramolecular heterodimer

2. Non-antibiotic biocides

An home-made on-line analytical apparatus to determine disinfectant residues in hospital warm water networks treated with ClO_2 for *Legionella* control

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Hospital-acquired legionnaires' disease arises from the presence of *Legionella* in hospital warm water distribution systems. The selection of an optimal method for water disinfection is a widely debated issue. Disinfection methods used by hospitals to control *Legionella* in warm water mainly include thermal eradication, hyperchlorination, chlorine dioxide treatment, copper-silver ionization, and point of use filters. In particular, chlorine dioxide is a strong disinfectant that has so far produced controversial results, more often due to the absence of an efficient system for the periodic determination of disinfectant residues in water.

In a previous study we randomly measured chlorine dioxide concentration in hospital warm-water distribution systems and we observed considerable fluctuations. These were mainly due to the variability of water flow during the day and to chlorine dioxide reduction determined by the presence of corrosion products and dissolved organic matter in water. Therefore, it was necessary to monitor the disinfectant concentration all day long at any remote point of each network to ensure the presence of ClO_2 at residual levels effective against *Legionella*. In order to afford this problem we have recently developed an automatic on-line apparatus capable to periodically collect and treat aliquots of warm water at the most strategic points of the examined networks.

During a measure cycle a fraction of warm water is made to flow into an airtight vertical manifold where a counter-current flow of compressed air continuously purges dissolved ClO_2 from water. The gas enriched in the analyte is piped in a second vertical manifold where bubbles into a flow of amaranth dye whose colour is continuously monitored by a UV/VIS detector. The decrease in absorbance at 522 nm is proportional to ClO_2 concentration. The optimization of this innovative equipment has required experimental tests for a thorough examination of the effect produced by the experimental conditions (water and reagent flow ratio, peristaltic pipe section, reaction pH and dye absorption wavelength) on the overall analytical sensitivity. Significant improvements have been obtained in the measure of aqueous ClO_2 residuals in the presence of commonly interfering species, such as ClO_2^-, ClO_3^-, NH_2Cl and Fe. This equipment has been installed in four Italian hospitals to monitor the residual concentration of ClO_2 used to disinfect warm water. The actual concentration of the disinfectant components was monitored every 90 min for at least 48 hours immediately downstream of the disinfection point and at the most remote distribution sites. A significant variability of disinfectant residues was observed during the monitoring activity and the fluctuations matched the variability of *Legionella* presence.

Keywords On-line analytical apparatus, chlorine dioxide, *Legionella*

Antifungal properties of Chlorhexidine digluconate and Cetylpirinidinium chloride on Oral *Candida*

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Introduction: The genus *Candida* forms part of the normal oral flora. With a low carriage rate of about 2% in the mouth of asymptomatic adults, the possible pathogenic role of *Candida* is usually underestimated. Several non-*Candida albicans Candida* (NCAC) are now known to be of major medical importance in humans in that they can contribute to opportunistic infections in patients with mucositis in the oral cavity. Incidence of candidal infection in the mouth is reportedly high in immune-compromised hosts as well as in neonatal and terminally ill patients. *C. tropicalis* and *C. krusei* are two examples of the NCAC that have emerged as virulent species. Both these have developed resistance to commonly prescribed azole antifungal agents. The use of mouth rinses with antimicrobial agents has been suggested as useful alternative to topical antimycotics. The objective of our study was to determine the minimal inhibitory concentrations (MIC) towards *C. tropicalis* and *C. krusei* of mouth rinses containing chlorhexidine (CHX), cetylpiridinium chloride (CPC) and the combination of CHX-CPC. We studied growth curves of *C. tropicalis* and *C. krusei* in the presence of mouth rinses to determine their mechanism of action as antifungal agent. *Methodology:* The MIC of the mouth rinses was determined using the broth dilution method. Both *Candida* sp. were then treated with the mouth rinses at the identified MIC values and their responses to the treatments were monitored periodically by measuring the changes in the turbidity of culture solutions to assess growth of the organisms. The growth curves of *C. tropicalis* and *C. krusei* following each treatment were plotted and the profiles were compared with those of the untreated organisms. The effect of each mouth rinse treatment on the morphology of the candidal colonies following an overnight incubation was also recorded. *Results and Discussion:* MICs of CPC were lower than those of CHX for both *C. tropicalis* and *C. krusei.* In the mixed formulation, CPC doubled the inhibitory effect of CHX towards both *Candida* sp., while CHX quadrupled the activity of CPC towards *C. tropicalis*. The growth curves showed profound suppression of growth for both *C. krusei* and *C. tropicalis* following treatment with all the three mouth rinses suggesting a fungicidal effect of CPC, CHX and CPC-CHX. The growth inhibitory effect of the mouth rinses was also observed by changes in the morphology of the growth colonies. They appeared coarse, wrinkle and dry after the mouth rinse treatments. Gargling using mouth rinses with such fungicidal activity would enhance a rapid reduction in the candidal population of patients with fungal infection. *Conclusion*: Antimicrobial mouth rinses incorporating CHX, CPC or their combination exhibited strong antifungal activities towards *C. tropicalis* and *C. krusei.*

Keywords *C. tropicalis; C. krusei;* chlorhexidine digluconate; cetylpirinidinium chloride

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Antimicrobial activity of silver nanoparticles synthesized by a new method

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Silver is known for its broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains, fungi, protozoa and certain viruses. Several researches have been focused on the potential antimicrobial activity of silver nanoparticles (AgNPs). Smaller nanoparticles due the larger surface-to-volume ratio have a more efficient antibacterial activity. In this work the antimicrobial effect of silver nanoparticles of 3,0 nm in size synthesized by a new method was evaluated against the Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 12228 and Gram-negative bacteria *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 15442, *Klebsiela pneumoniae* ATCC 10031 e *Enterobacter aerogenes* ATCC 13048 by the agar diffusion test. We prepared colloidal silver nanoparticles by a simples and quickly method by using silver sulfadiazine and a cationic surfactant at the critical micellar concentration (1 x CMC). It was observed that the colloidal silver nanoparticles showed the higher antimicrobial effect ($p<0.05$) against *P. aeruginosa e E. coli* than the chemical reagents used in the synthesis.

Keywords: silver nanoparticles, antimicrobial agents, cationic surfactant

Antimicrobial effect of a nitric-oxide-gas-releasing solution on surface and dermal pathogens

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Introduction: Prior to surgery or other invasive procedures skin must be treated with topical antimicrobial products to prevent nosocomial infections by reducing the number of microorganisms on the skin. Unfortunately, there is an increase in drug resistance to these products and there is now a need to explore other agents. Nitric oxide is a simple molecule produced in the body which acts as a non-selective broad range antimicrobial which is key to the innate immune system. Our previous work has shown that gaseous nitric oxide is an effective broad spectrum antimicrobial agent which is well tolerated and unlikely to develope microbial tolerance. We demonstrate, in an *in vitro* model, that a nitric oxide releasing solution has 100% cidal action within 10 minutes against *Escherichia coli, Acinetobacter baumanii,* Methicillin-Resistant *Staphylococcus aureus* (MRSA) bacterial strains and Influenza A/Victoria/3/75 (H3N2), A/Denver/1/57 (H1N1 and H3N2) and B/Hongkong/5/72 viral strains. We further provide data that suggests that this compound is not toxic to sensitive monocytic cell line THP-1 cells.

Methods: A novel nitric oxide releasing solution (NORS) was donated (NSI Inc., Canada). Thirty µl (of 10^6 cfu/ml) were added to 2.7 mL of either sterile saline or NORS at strength A, B, C or D, in each well of a six well plate to attain 10^5 cfu/mL. Samples of 1,10 and 100µl at various time points, up to ten minutes, were taken and plated on agar plates and grown at 37^0 C O/N. Aliquots of 20 µL virus (x 10^5 plaque forming units, pfu), diluted in PBS, were spotted onto a sterile glass surface, spread into a film and allowed to dry for15-20 min. Each sample received two sprays (200 uL) separated by 5 minutes of concentrations A, B, C, D of NORS or of normal saline. After 5 min, all samples were reconstituted in 1.0 mL PBS and assayed by standard plaque formation technique in appropriate cells. Monocytic cell line THP-1 were appropriately cultured to a cell density of 3 x 10^5 per well in a 96 well plate, and incubated with escalating doses of NORS and subsequently stained with propidium iodine to be measured with flow cytometry for cell viability. All measurents were done in triplicates.

Results: All bacterial strains had less than 6% viability using solution B, 2 log reduction when solution C was added and complete eradication using solution D. Time was shown to play a major role in eradication of bacteria by nitric oxide releasing solution. All *A. baumanii* was eradicated after 5 min using solution D, while 8 min were required for *E. coli* and 10 for MRSA. NORS concentrations of B,C and D prevented plaque formation in both strains of Influenza A, whereas at concentration A, there was a 2 $\log_{10}$ reduction in pfu/mL. Influenza B was less sensitive to NOD, yet at concentration D, 100% of the virions were inactivated. The toxicity of NORS to THP-1 cells based on 50% cell death (LD_{50}) was approximately 5 times concentration D.

Conclusions: Together, these results suggest that nitric oxide releasing solutions might be useful as topical antiseptics or utilized as skin preparations to remove potential microbes that contribute to nosocomial infections. It also appears that this solution may be non-toxic to host cells. Further studies in this area is warranted.

Keywords Nitric oxide; bacteria;nosocomial; Antimicrobial/Bacteriocidal

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Antimicrobial effect of silver nanoparticles in controlling bacterial adhesion to stainless steel

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Due to the increase in the number of bacteria resistant to antibiotics, researches with silver, particularly as nanoparticles, have been increased in the last years. Moreover, silver is the metal which has the lowest toxicity to animal cells. In this context, this work presents as objective to evaluate the antimicrobial effect of silver nanoparticles (SN) obtained by a new method of synthesis proposed by Fernandes (2010) against gram-positive and gram-negative strains as well to assess the ability of these nanoparticles to remove vegetative cells adhered to stainless steel surfaces. The new technique for obtained SN is simple and rapid and involves an auto-oxirreduction of the silver sulfadiazine catalyzed by presence of the surfactant dodecyltrimethylammonium (Dotab) micelles. We observed that the SN was efficient for removal of cells of *P. aeruginosa* adhered on stainless steel coupons (1.0 mm x 1.0 mm x 0.1 mm), reaching 5 decimal reductions in the bacterial population after 30 min of the contact. The performance of the SN was also better when compared to sodium carbonate solution and water in removing cells from *B. cereus* on stainless steel cylinders (336,5 cm^2). We suggested, therefore, the application of silver nanoparticles solutions obtained by this synthesis as an antimicrobial using, for example, in the sanitization of food contact processing surfaces particularly of utensils.

Keywords: silver nanoparticles; antimicrobial, food industry; sanitization

Bacterial Contamination of Emergency Department Ultrasound Equipment

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Purpose: Ultrasound is an efficient, noninvasive diagnostic tool, which has gained widespread use in the emergency department for the evaluation of medical conditions and to assist in invasive procedures. A recent emergency department methicillin resistant staphylococcus aureus (MRSA) abscess study required enrollees to undergo ultrasound evaluation of the abscess cavity, thus, increasing the overall use of ultrasound in the ED. This comparative study determined if pathogens contaminate the ultrasound machine during increased use of bedside ultrasound.

Methods: Culture swabs were taken from three ultrasound machines located in the ED: Two in the main ED and the third in the trauma bay. No modification of the cleaning schedule was done during the study period representing typical use. Sample cultures were taken during a three week period. Each machine was sampled at three different sites: Keyboard/controls, transducer tip and transducer handle. At the start of the study initial swabs was taken before through cleaning of the ultrasound machine to determine preliminary antimicrobial contamination. Subsequent cultures were taken at one and three weeks after this cleaning. The samples were sent to the microbiology lab for analysis.

Results: Prior to through cleaning of the machine, there was mild bacterial growth on the keyboard/controls. One of the transducer probe handles was colonized with MRSA and the transducer tip had mild bacterial contamination. After one week two out of the three transducer tips had mild bacterial growth and one transducer handle was mildly contaminated. On the third week the keyboard/controls of all machines had moderate to heavy bacterial growth, all of the transducer handles had mild to moderate growth, and the transducer tips had mild bacterial growth on two of three machines. There was no MRSA isolated.

Conclusion: Emergency department ultrasound machines are contaminated on areas where the operator interfaces with the equipment especially the keyboard and controls. This contamination is due to incomplete cleaning and transferring bacteria from the patient to the machine. In order to avoid the introduction of harmful pathogens between patients, clinicians must be vigilant to properly clean all areas of ultrasound equipment before and after each use.

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Effect of peracetic acid on *Bacillus cereus* spores evaluated by phase contrast microscopy and fluorescent probes staining

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B. cereus group exhibit highly divergent ecological and pathogenic properties. Due to its capacity to form resistant spores and its prevalence in nature and foodstuff, it is recognised as an important pathogen and spoilage microorganism in food processing industry. Peracetic acid is an oxidizing agent which shows a sporicidal activity and is commonly used in food industry for surface and material sanitation. The aim of this study was to use phase contrast and fluorescent microscopy to evaluate the effect of peracetic acid treatment on spores of the psychrotolerant strain KBAB4.

A treatment of 0.85g/l acid peracetic has been applied at 20°C to a population of 10^6 spore/ml. After acid neutralisation, the efficiency of the treatment has been classically evaluated by enumeration of the survivors on agar plates. In parallel, microscopic observations of individual spores with and without fluorescent staining have been made with the "Live Dead" kit (DMAO and EthD-III, Interchim); DAPI, INT and acridine orange. Non treated spore suspensions presented 3 types of spores (I, II and III) with type I being the most representative, *i.e.* intact spores that appeared bright under light microscope with low DMAO staining at the boundary. Weak staining of intact spores was expected as previously reported by some authors (Laflamme *et al*., 2004). While few differences were observed directly after disinfectant treatment, later alterations appeared more important and cell shape damages were observed both with phase contrast microscopy and with partial entry of EthD-III. Microscopic observations done 4 hours and 44 hours after acid peracetic treatment show loss of viability and greater permeability to fluorescent compounds. Figure 1 presents the 5 types of spores observed after peracetic acid treatment.

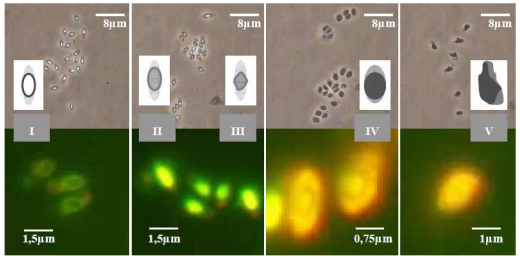

Figure 1 Microscopic observations of spores after treatment with peracetic acid in phase contrast microscopy (upper line) and fluorescent microscopy (lower line). Based on spore shape and viability observed after staining with DMAO and EthD-III, 5 types of spore have been mentioned.

After 4 hours treatment, 80% of spores have been enumerated on agar medium and could be correlated to 70% of spores appearing as intact (35% type I) and viable (35% type II and II) with fluorescent staining as opposed to the 30% non viable spores (type IV). After 44 hours, no survivor were culturable on agar medium while viability or membrane integrity was observed for 62% spore, *i.e.* 21% type I and 41% type II-III. Spore inactivation is a complex phenomenon and the use of fluorescent compounds may offer a good alternative to detect viable but non-culturable cells and provide further information on physiology and viability of individual stressed cells after a disinfectant treatment.

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Keywords viability; germination; membrane integrity; physiology; food-borne pathogen.

Effects of antibacterial chemicals on *Bacillus cereus* attached to different surfaces

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Common food surfaces are made of stainless steel, plastic material or glass. These can be cross-contaminated by microorganisms associated with food or microorganism frequently found in the kitchen area. One of the causes to foodborne illness is *Bacillus cereus*. Some strains produce enterotoxins that may cause diarrhea or emetic syndrome and that is especially critical in hospital environments. The vegetative cells of *B. cereus* can be significantly reduced by heat and time (e.g. 65°C for 5 minutes) at high pH ≥ 10 (1). However, the endospores survives at higher temperatures, without nutrition or moisture and when they reach a suitable environment, vegetative cells are produced.

The purpose of this study is to evaluated the adhesion of *B.cereus*, (ATCC 14579) vegetative cells and endospores, on three different types of material; plastic (polyoxymethylene, POM, approved by FDA for use in the food industry), glass and stainless steel. Furthermore the reduction of *B.cereus*, vegetative cells and endospores on theses surfaces was evaluated for commonly used cleaning chemicals, chlorine and peracetic acid and compared to a selected mixture of amino acids.

The invstigation was performed on spheres with a diameter of about 3 mm. The amount of attached *B.cereus*, vegetative cells and endospores on the different materials was analysed. A special analysing method was developed to evaluate the amount of *B.cereus*, vegetative cells and endospores, respectively, based on heat treatment of the withdrawn samples.

Our experimental results show that cells and endospores adhere stronger to plastic spheres than to glass or stainless steel spheres. When a second washing of the spheres with pure water were conducted the reduction of *B.cereus* was larger on glass and steel spheres than on plastic spheres. The different chemicals functions all as biocidal agents, but acts differently on the microorganism and has different capacity to reduce vegetative cells and endospores of *B.cereus*.

Keywords adhesion; antimicrobial, surfaces, *Bacillus cereus*

Reference:

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Electrochemically activated solutions; evidence for antimicrobial efficacy, optimal storage conditions, and potential applications of this novel multi-purpose biocide.

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This study provides an overview of the scientific evidence for the mode of action, antimicrobial spectrum, and potential applications of Electrochemically Activated Solutions (ECAS). Due to the problems associated with the use of existing biocidal agents there is a need to explore new methods of disinfection to help maintain effective bioburden control, particularly since legislation and the prohibitive cost of development have significantly reduced the number of biocide products available. The transformation of low mineral (NaCl) solutions into an activated metastable state, by electrochemical unipolar action, produces a solution (ECAS) containing a variety of oxidants including hypochlorous acid, free chlorine, and free radicals known to impart antimicrobial properties.

European standard suspension tests (EN:1040, EN:1276, EN:14347, EN:13704 & EN:13623) were used to assess the antimicrobial activity of ECAS, whereby it was shown to be an effective bactericidal and sporicidal agent under defined controlled laboratory conditions. However, in agreement with existing scientific knowledge and theory, it was observed that organic soiling/loading significantly impacts on the antimicrobial potential of this biocide. Long term storage tests were conducted, whereby ECAS was stored within glass or plastic universal tubes, at 4°C or 20°C, and the physicochemical parameters (pH, ORP & free chlorine) and antimicrobial efficacy (according to EN:1040) tested at regular intervals. The results indicated that the stability of ECAS is dependent on both vessel material and temperature, with maximum stability being obtained when stored at 4°C in glass, although all storage conditions yielded solutions retaining significant antimicrobial activity even after 100 days.

Surface disinfection studies were carried out using *Bacillus atrophaeus* endospores applied to various surface materials (25mm^2), either used in the assay immediately (wet spores) or left overnight to dry before the assay (dry spores). Contaminated surfaces were exposed to either 500µl of ECAS or an appropriate control solution (Ringer's), using single or double dosing, for 15, 30 or 60 minute exposure times. ECAS treatment elicited a significantly reduction in the number of viable recoverable spores for all treatment surfaces compared to controls. As expected, increasing the dose or treatment duration increased the efficacy, although there were clear differences in the percentage reduction in viable recoverable spores among treatment groups. In general, dry spores were significantly more resistant than wet spores, and non-porous surfaces were more effectively decontaminated than porous surfaces (except raw steel).

ECAS has been shown to have broad-spectrum antimicrobial activity, even after 100 days storage (under defined conditions), and has the ability to decontaminate inanimate surfaces. Due to low cost raw material requirements and ease of production (either remotely or *in situ*), ECAS could be used as an effective decontaminant in a variety of settings. Moreover, ECAS produces no known toxic by-products, reverting to an inactive low mineral (NaCl) solution, and therefore there is no bio-hazard associated with its usage. Numerous studies have found ECAS to be highly efficacious in practice, as both a novel environmental decontaminant and within the healthcare environment as a topical treatment agent (with reported low accompanying toxicity), and our experimental *in vitro* data supports these studies. However, the use of ECAS still remains confined to limited applications in a small number of countries, and further scientific research is required to accurately model the spatial and temporal disinfection process of these fast acting biocides.

Keywords: Electrochemically activated; biocide; decontamination.

Enhancement of the antimicrobial performance of biocidal formulations used for the preservation of White Mineral Dispersions

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White Mineral Dispersions (WMD: ground and precipitated calcium carbonate, modified calcium carbonate, clay, and talc) are water-based mineral dispersions used as filler and pigment in the paper and paint industries. Although the alkaline pH of WMD ranges from 8 to 10, and the water content from 25-80 % (w/w), the presence of various salts and the available oxygen are sufficient to promote an adequate microbial propagation environment. Bacterial contamination of WMD often leads to acidification of the product and hence to changes in the dispersion stability in terms of viscosity. Therefore, biocides play an important role in the preservation of WMD in order to maintain high quality and hygiene standards, such as brightness, rheological parameters as well as odour neutrality. Due to the occurrence of biocide-resistant bacteria and technical limitations in the use of biocides, new preservation strategies are required – like the enhancement of biocides by non-biocidal compounds. The aim of this study was to evaluate the biocide enhancement performance of lithium against biocide-resistant *Pseudomonas* sp. in WMD. Subsequently, the minimal enhancer concentration (MEC) of lithium and the bioavailability of lithium in respect to the mode of introduction into WMD were investigated. The antimicrobial performance of biocidal formulations comprising isothiazolinones and formaldehyde-releasers or isothiazolinones and glutardialdehyde has been evaluated against the related resistant bacterial spectrum in the presence of lithium. The minimal enhancing concentration (MEC) of lithium ranged from 1350 ppm to 1500 ppm (based on the liquid phase weight of a WMD with 75% solids) for formaldehyde-releasers and glutardialdehyde-based biocidal formulations, respectively. The biocide enhancing property of lithium was independent of whether lithium was introduced into WMD via a lithium-neutralised dispersant, added during the calcium carbonate grinding step, or dosed into the final product. Lithium is a non-biocidal compound which has been discovered to be a potent and universal biocide enhancer. Lithium boosts the biocidal activity of various biocides and provides a novel technique to overcome biocide resistance in WMD. This approach may solve the emerging problems of bacterial resistance or adaptation by using an enhancer compound to assure the effectiveness of the biocide. Such a biocide enhancer represents a breakthrough that offers a potential tool to revolutionise the consumption of biocidal agents in the WMD producing industry.

Keywords biocide, enhancement, resistance, bacteria, lithium, calcium carbonate slurry, white mineral dispersion

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Evaluation of biocidal activity of Evolyse, a disinfectant based on hydrogen peroxide and silver nitrate

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The aim of this study was to test the *in vitro* biocidal activity of a novel disinfectant, Evolyse, based on hydrogen peroxide (5.9 %) and silver nitrate (10 ppm) against a wide spectrum of microorganisms, including bacteria, spores and fungi. *In vitro* activity was determined using the quantitative suspension tests described by the European Committee for Standardization. The antimicrobial efficacy of disinfectant delivered by a nebulising device was also evaluated under practical conditions, in different hospital environments (operating room, sterilization room, dental office) by microbiological monitoring of air and surfaces, before and after disinfection treatment.

The disinfectant exhibited bactericidal activity (reduction factor $>10^5$) against *Staphylococcus aureus* ATCC 25923, methicillin-resistant *S. aureus* strain, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 25922, *Salmonella* Enteritidis ATCC 13076, *Pseudomonas aeruginosa* ATCC 27853 and *Legionella pneumophila* ATCC 33152 after 5 min contact at room temperature. Sporicidal activity against *Bacillus cereus* ATCC 11178 spores (reduction factor $>10^3$) was obtained after 45 min contact at room temperature. The disinfectant also showed fungicidal activity (reduction factor $>10^4$) against *Aspergillus flavus* ATCC 46283 and an *Aspergillus niger* wild strain after 15 min contact at room temperature and against *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 2601 and *Penicillium chrysogenum* ATCC 9179 after 30 min contact time. The presence of interfering substance to simulate practical conditions (bovine albumin, final concentration 0.3 and 0.03%) did not diminish the antimicrobial activity in any of the strains tested.

Microbiological monitoring of the air and surfaces in the hospital environments, before and after disinfection treatment, showed a percent reduction of bacteria and fungi higher than 70% in the majority of microbial samples carried out in the operating room, sterilization room and dental office.

In conclusion, the results of this study showed the very good biocidal activity of Evolyse and offer promising perspective for the use of this product in hospital environments.

Keywords Evolyse; antimicrobial activity; hospital environments

Incidence of biocide resistance among *Enterococcus faecalis* and *E. faecium* strains

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Introduction. Biocides are commonly used in many different settings, eg. as sanitizers in the food industry, in cosmetics, and for disinfection in hospital settings, among other uses. Bacteria challenged with biocides may acquire or develop biocide resistance mechanisms. Failure of biocide treatments due to microbial resistance is a threat when food spoilage or human pathogenic bacteria are involved in the resistance. Enterococcci are a bacterial group attractive for investigation of biocide resistance, since these bacteria are commonly found in foods, water, and the gastrointestinal tract of warm-blooded animals. In the last decades, enterococci have become well-recognized human pathogens due to the acquisition of virulence traits and antibiotic resistance. Enterococci may also act as reservoirs of antimicrobial resistance in foods. The aim of this study was to evaluate the levels of resistance to commonly used biocides among enterococci isolated from different sources (including foods, animal feces, and clinical sources).

Material and methods. A total of 285 strains (including *E. faecalis* and *E. faecium* species) isolated from meat and dairy products (n=40), seafood (n=25), vegetable foods (n=32), wild flowers (n=47), animal feces (n=82) and hospitals (n=59) were tested for resistance levels to the following biocides: benzalconium chloride (BC), hexadecylpyridinium chloride (HDPC), chlorhexidine (CH), cetrimide (CT) and triclosan (TC). MICs were determined by the broth micro-dilution method in 96-well microplates as described by the Clinical and Laboratory Standards Institute (CLSI) after 24 h incubation at 37ºC.

Results and conclusions. All isolates were sensitive to triclosan (MIC 0.0025%), and also to cetrimide (MIC 0.025%) with exception of a small percentage (3.39%) of clinical isolates (MIC 0.025%). Sensitivity to benzalconium was also very high (MIC 0.0025% for all isolates from seafood, vegetable foods and animal feces, and also in 82% to 97% of the rest of isolates). Most isolates from food and animal sources were also very sensitive to chlorhexidine (MIC 0.0025% for 52 to 65% of isolates, and MIC 0.025% for 17 to 44% of isolates). Clinical isolates showed relatively higher MICS, of 0.025% for 60% of isolates. Results from this study indicate that enterococci from foods do not exhibit particularly high levels of resistance to common use biocides.

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Keywords: biocides; enterococci; foods.

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Increased resistance to detergent in *Enterococcus faecalis*

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Enterococcus faecalis is a gram-positive bacterium found in water, soil, vegetation and food, and present in the gastrointestinal tract of a variety of organisms including humans. Considered harmless to human health for a long time, *E. faecalis* has since emerged as a leading nosocomial pathogen and as an infectious agent in endocarditis, meningitis, endophthalmitis and other serious diseases. Besides its sturdiness and high adaptability to environmental changes, *E. faecalis* often displays resistance to various antimicrobial agents and has been reported to survive prolonged stay on environmental surfaces. This prompted us to research *E. faecalis* tolerance to detergents, specifically sodium N-lauroylsarcosinate (sarkosyl) and sodium dodecyl sulfate (SDS) that are routine household chemicals.

A clinical isolate of *E. faecalis* that exhibited plasmid-mediated resistance to tetracycline and kanamycin was used in this study. The strain was grown in the presence of increasing concentrations of sarkosyl or SDS, and its sensitivity to these two anionic detergents was compared to that of two *Escherichia coli* strains, HB101(pBR322) and HB101 (pBR325). The minimum inhibitory concentration (MIC) of sarkosyl and SDS against the strains was the minimum concentration required to observe no growth after 30 h culture at 37°C, with aeration, in Luria broth (LB) medium.

Results showed that *E. faecalis* was much more sensitive than *E. coli* to both detergents with the MIC of sarkosyl being 100 times smaller for *E. faecalis* (0.1%) than for *E. coli* (10%) and that of SDS being 120 times smaller for *E. faecalis* (0.05%) than for *E. coli* (6%). Growth of *E. faecalis* in the presence of 0.06% sarkosyl led to the isolation of a mutant strain that exhibited accrued resistance to the detergent with a MIC (0.6%) that was 6 times higher than the original value. Besides accrued resistance to sarkosyl, the isolated mutant grew faster in LB medium than the original strain (the lag period was reduced from 4 h for the control to 2 h for the mutant) and reached a higher cell density at stationary phase. The mutant did not exhibit accrued resistance to SDS; it remained resistant to tetracycline and kanamycin although resistance to the latter was reduced, with MIC dropping from 2000 µg/ml to 500 µg/ml. This was not due to plasmid loss as plasmid DNA preparations from the mutant were identical to those of the original strain after electrophoresis in agarose gels and would confer resistance to either antibiotic after transformation of *E. coli* strain HB101. Similarly to the original strain, the resistance to kanamycin and tetracycline in the mutant was carried on two small plasmids (~ 7 kb and 5.7 kb). The mutant strain also presented the characteristics of an *E. faecalis* strain, namely that it was gram-positive, exhibited no catalase activity, was able to grow in the presence of 6.5% NaCl as well as at pH 9.6, and would grow on bile-esculin agar with black discoloration of the agar.

E. faecalis has been known to survive lethal concentrations of detergents such as bile salts or SDS after brief exposure to homologous sublethal concentrations, with strong cross-resistance observed with bile salts- and SDS-adapted cells. Here we show the occurrence of a mutant strain with accrued resistance to sarkosyl, another anionic detergent, after growth in half the detergent MIC. While SDS is a common component in household cleaning products, sarkosyl is used as a foaming and cleansing agent in shampoo, shaving foam and foam wash products. Although the mutant did not exhibit cross-resistance to SDS, it displayed characteristics that gave it advantages over the original strain. Interestingly the mutant retained the two plasmids present in the original strain although we had previously shown that sarkosyl is a potential curing agent for the strain. The findings reported here further emphasize the remarkable capacity of *E. faecalis* to resist harsh treatment and the potential emergence of strains with increasing tolerance to detergents commonly used for cleaning purposes in households.

Keywords *Enterococcus faecalis*; *E. coli*; sarkosyl; SDS; antibiotics resistance; plasmids

Influence of temperature of the dipping solution on the antimicrobial effectiveness of acidified sodium chlorite (ASC) against pathogenic bacteria on poultry

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Background: Little information is available regarding the influence of the temperature of the solutions in the antimicrobial effectiveness of poultry decontaminants. In this research three temperatures (4, 20 and 50º C) of acidified sodium chlorite (ASC) solutions were compared for their effectiveness to reduce loads of pathogenic bacteria on poultry throughout storage, in order to identify the best temperature of application for the decontaminant evaluated.

Methods: A total of 120 chicken legs were collected from a local poultry processing plant immediately after evisceration. Samples were randomly divided into six groups, each containing 20 legs. Samples in each group were inoculated with *Listeria monocytogenes* NCTC 11994, *Staphylococcus aureus* ATCC 23235, *Bacillus cereus* ATCC 21769, Salmonella enterica ser. Enteritidis CECT (Spanish Type Culture Collection) 556, *Escherichia coli* ATCC 12806 and *Yersinia enterocolitica* NCTC 11174, respectively. Fifteen samples in each group were dipped for 15 min into 500 mL of sterile solutions (w/v) of sodium chlorite (1,200 ppm; Fluka, Madrid, Spain) acidified to pH 2.7 by adding citric acid (Panreac, Barcelona, Spain). Three different temperatures of dipping solutions were used in each group: 4º C (5 legs), 20º C (5 legs) or 50º C (5 legs). Five legs in each group weren't treated (control). After the treatments, the chicken legs were drained for 15 min at 20º C. The samples were individually placed in sterile bags and stored at 4º C for 5 days. The microbiological quality was determined after 0, 1, 3 and 5 days of storage. On day 0 the legs were tested immediately after the inoculation and dipping treatment had been completed. Each sample was prepared by excising 5 g of skin with a sterile knife blade. The samples were placed in a sterile stomacher bag containing 45 mL of sterile 0.1% (w/v) buffered peptone water (Oxoid Ltd.) and homogenized (Masticator IUL, Barcelona, Spain) for 2 minutes. Serial dilutions in sterile 0.1 % (w/v) buffered peptone water were prepared from this homogenate, and aliquots of 0.1 mL were surface plated in duplicate onto plate count agar (PCA, Oxoid Ltd.) and incubated for 48 h at 25º C (*B. cereus* and *Y. enterocolitica*) or 35º C. Data obtained were compared for significant differences ($P<0.05$) using an analysis of variance (ANOVA) and the Duncan test.

Results: Average reductions (related to the control samples) by ASC during storage ranged from 0.25±0.56 $\log_{10}$ cfu/g (*L. monocytogenes*; 50º C) to 2.18±1.01 $\log_{10}$ cfu/g (*E. coli*; 20º C). Higher ($P<0.05$) average reductions were observed in Gram negative (1.01±0.98) than in Gram positive (0.68±0.71) bacteria. Analysis of variance showed that the temperature play a role in bacterial reductions by ASC. The reductions achieved at 4º C (average: 0.60±0.71 $\log_{10}$ cfu/g; range: 0.43±0.95 to 1.04±0.64 $\log_{10}$ cfu/g) and 50º C (average: 0.67±0.71 $\log_{10}$ cfu/g; range: 0.25±0.56 to 0.95±0.86 $\log_{10}$ cfu/g) were lower ($P<0.05$) than those obtained at 20º C (average: 1.25±1.01 $\log_{10}$ cfu/g; 0.92±0.64, 0.89±0.62, 0.78±0.56, 2.11±0.87, 2.18±1.01 and 1.83±1.15 $\log_{10}$ cfu/g for *L. monocytogenes*, *St. aureus*, *B. cereus*, *S. enterica*, *E. coli* and *Y. enterocolitica*, respectively).

Conclusion: Findings in the report being presented here suggest that the temperature of the dipping solution is an important factor to be considered when ASC is applied for decontamination of poultry.

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Keywords: poultry, decontamination, acidified sodium chlorite, temperature of treatment, *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Salmonella enterica, Escherichia coli, Yersinia enterocolitica.*

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Kinetics of inactivation of *Klebsiella pneumoniae* adhered to lettuce with organic chlorine compound and vinegar

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This study used the HACCP based approach and aimed to evaluate the efficiency of 170 $mg \cdot l^{-1}$ free available chlorine (FAC), expressed by Cl_2, pH 6.0, prepared from sodium dichloroisocyanurate and 0.18 % acetic acid pH 2.78, prepared from comercial vinegar in the reduction of the populations of *Klebsiella pneumoniae* adhered to lettuce leaves surface as a function of contact times. For treatment using chlorine compound, the contact times were of 30 s and 1, 2, 3, 4, 5, 8, 16, 24, 32, 40, 48, 56 and 64 min and for treatment using acetic acid were 5, 10, 15, 20, 25, 30, 40, 50 and 60 min. To describe the kinetics of inactivation of *K. pneumoniae* adhered to the lettuce intact leaves, the log $cfu.g^{-1}$ of the survivors after sanitizers actions was evaluated by using the two-term exponential model for mixed cell population, according to the Pruitt and Kamau. The initial population of *K. pneumoniae* in the leaves of letuce were aproximately 6 log $cfu \cdot g^{-1}$. The value of the surviving fraction of the population most sensitive (f) and the specific rate of death for sensitive subpopulations (b_1) and resistant subpopulations (b_2) were obtained by nonlinear regression using the Statistical Analysis Systems, version 9.1. The D value was measured from the linear portion of the curve using the equation $D_i=2{,}303/b_i$, where i is the fraction sensitive or resistant population of *K. pneumoniae*. Was observed in the heterogeneous population the prevalence of sensitive fraction ($f = 0.9647$) that was reduced to a high specific rate ($b_1 = 3.2018 \cdot min^{-1}$). The resistant fraction of the population was reduced to a low specific rate ($b_2 = 0.0357 \cdot min^{-1}$). The D value predicted for the sensitive subpopulation was 43 s and the resistant subpopulation was 64.5 min. The population of *K. pneumoniae* using the vinegar solution with low concentration of acetic acid was reduced at lower rates and more slowly than treatment with the organic chloramine. The sensitive fraction was prevalent ($f = 0.7644$) and was inactivated at a low specific rate ($b_1 = 0.5456 \cdot min^{-1}$) and the resistant fraction of the population was reduced at very low specific rate ($b_2 = 0.0187 \cdot min^{-1}$). The D value predicted for the sensitive population was 4.22 min and the resistant subpopulation was 124.6 min. The efficiency of the treatments was dificulted due to inaccessibility of products to reach the cells in microenvironments on the lettuce surface. Furthermore, the surface hidrophobicity and roughness can influence the efficiency of the sanitization procedures. Results provide insights to predicting the efficiency of sanitizers in reducing populations of *K. pneumoniae* and others microorganisms in lettuce leaves.

Keywords: Survival models, *Klebsiella pneumoniae*, lettuce

Legionella pneumophila isolation rate in a Spanish hospital pre- and post-installation of an electro-chemical activation system for potable water disinfection

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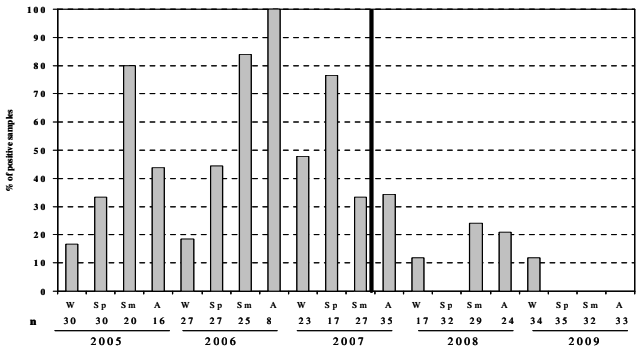
Objective: Hospital-acquired legionnaires' disease arises through potable water. This study explores the efficacy of an electro-chemical activation system for *Legionella* disinfection of potable water systems.

Methods: A retrospective analysis of data on Legionella isolation obtained in the routinely monthly surveillance performed at Residencia Cantabria (Santander, Spain) by BSA was performed. Data of all 521 samples (250 pre- and 271 post-installation) collected always from unfiltered taps from the potable hot water system from 1/January/2005 to 31/December/2009 was considered. Water was collected and processed for legionella isolation, and temperature and chlorine measured. The water disinfectant apparatus (Hydrostel; Idrovital S.r.l, Empoli, Fi, Italy) was installed in the inflow of water-heating tanks on 23/September/2007 and consisted in an automatic system for the production of an aqueous solution of NaOCl from water and NaCl (99.5% purity) through an electrolysis procedure (Electro Chemical Activation System). Differences between isolation rates were analysed by the chi-square or Fisher's exact tests. A step-wise logistic regression analysis was performed to predict Legionella isolation.

Results: Chlorine levels were higher (p<0.001) post-installation (0.55±0.41 vs. 1.21±0.38 ppm). A total of 28.2% (147/521) samples were positive for *L. pneumophila*; 112 (76.2%) of them serogroups 2-14. Post-installation, the isolation rate was lower (46.8% vs. 11.1%; p<0.001; OR=0.14; 95%CI=0.09-0.22) due to significant reduction in *L. pneumophila* serogroups 2-14 from 38.4% to 5.9% (p<0.001; OR=0.10; 95%CI=0.06-0.18). In the step-wise multivariate analysis, the installation of the system was the main variable associated with the reduction of Legionella isolation (p<0.001; OR=0.26; 95%CI=0.15-0.46). The figure shows per-season (W: winter, Sp: spring, Sm: summer, A: autumn) no. and % of positive samples in pre- and post-installation periods (black vertical line: installation time).



Conclusion: The electro-chemical activation system was efficacious in significantly reducing over time isolation rates by significantly increasing chlorine levels. This may have clinical relevance with respect to hospital-associated legionellosis since *L. pneumophila* serogroups 2–14 is not detectable by the urinary antigen test.

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Modulation of Chlorhexidine antimicrobial activity and cellular citotoxicity by the inclusion in different cyclodextrin

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Chlorhexidine (Cx) is widely used as antiseptic and therapeutic agent in medicine and dentistry. Its inclusion compounds have been developed for use in controlled release. The citotoxicity in bacteria and eukaryotic cells of Cx-cyclodextrin compounds is still unknown. The aim of this study was to prepare supramolecular complexes of Cx and to alfa-cyclodextrin (α-cd), beta-cyclodextrin(β-cd) and hydroxypropyl-beta-cyclodextrin (Hpβ-cd) using freeze-drying method at different molar ratio (1:1; 1:2; 1:3 and 1:4) to evaluate their antimicrobial activity, membrane interactions and citotoxicity on cell culture. The Inhibitory Concentration (IC) was determining to Streptococcus mutans (S.m.), Candida albicans (C.a) and Aggregatibacter actinomycetemcomitans (A.a). Characterization of the mechanism of interaction between these compounds with the yeast membrane and assessment of membrane morphology were performed using SQM (Sterol Quantification Method). Cell viability was determined by Neutral Red assay, a colorimetric method for determining the number of viable cells. To this purpose, single cell suspension of osteoblasts of a primary culture or fibroblasts and were treated with 0.1; 0.01 and 0.001% concentrations. The optical density (OD) of samples was measured at 540 nm.

The IC values found varied between 4- 2 µg/ mL for Cx:α-cd, 4- 0.5 µg/ mL for Cx:β-cd, 4- 2 µg/ mL for Cx:Hpβ-cd inclusion compounds against C.a. To S.mutans the IC values found varied between 16- 8 µg/ mL for Cx: α-cd and Cx:β-cd, 16- 2 µg/ mL for Cx:Hpβ-cd. To A.a the IC values found varied between 8- 4 µg/ mL for Cx:α-cd, 8-0.5 µg/ mL for Cx:β-cd, 8- 2 µg/ mL for Cx:Hpβ-cd.

SQM analysis revealed that ergosterol solubilization increased proportional to cyclodextrin concentrations: Cx: α-cd (56.13; 88.57; 146.15; 160.75%), Cx:β-cd (78.07; 129.82; 125.73; 117.54%), Cx:Hpβ-cd (79.82; 108.16; 114.04; 112.87%) for 1:1, 1:2, 1:3, and 1:4 respectively, likely as a consequence of membrane lipid solubilization.

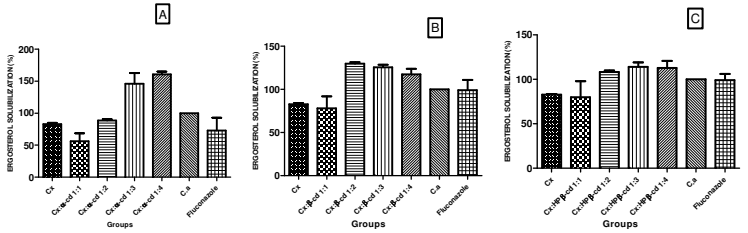

Figure 1. Percentage of ergosterol solubilization *Candida albicans* membrane treated with Cx: α-cd (A), Cx:β-cd (B), Cx:Hpβ-cd (C)

The cell viability tests showed that 0.1% of all inclusion compounds and Cx were severely citotoxic while the 0.01 and 0.001% compounds showed mild citotoxicity to moderate. The more severe degrees of citotoxicity (< 30%) were observed for Cx:α -cd compounds, Cx: α-cd 1:2, Cx:β-cd and Cx:Hpβ-cd 1:3 proved to be slightly citotoxic (60-90%) while controls were able to cds pure will stimulate cell proliferation (>100%). The osteoblasts are more sensible to chlorhexidine effects generally. In the eukaryotic cells the lowest citotoxicity were founded after high proportion of CD was used (1:2 and 1:3 molar ratio), and the pure cyclodextrins were able to stimulate osteoblasts and fibroblasts proliferation. All compounds were more citotoxic to osteoblasts than fibroblasts. The best ergosterol solubilization founded in Cx:cd groups would be justify the most severe citotoxicity observed in these compounds.

In conclusion cyclodextrins were able to modulate the Cx supramolecular compounds antimicrobial activity and the biocompatibility.

Keiwords: cyclodextrins, slow delivery system, chlorhexidine

Molecular mechanisms of chlorhexidine tolerance in *Burkholderia cenocepacia* biofilms

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The high tolerance of biofilm-grown (sessile) *Burkholderia cepacia* complex bacteria against antibiotics and disinfectants presents considerable problems in several areas, including the treatment of infected cystic fibrosis patients and the implementation of infection control guidelines.

In the present study we compared the tolerance of planktonic and sessile *Burkholderia cenocepacia* J2315 cultures and examined the transcriptional response of sessile cells to treatment with chlorhexidine, using microarrays and qPCR.

At very low (0.0005%) and very high (0.05%) concentrations, chlorhexidine had a similar effect on both sessile and planktonic populations, but at intermediate concentrations (0.015%), antimicrobial activity was much more pronounced in planktonic cultures cells than in biofilms. The exposure of sessile cells to chlorhexidine resulted in an upregulation of the transcription of 469 (6.56%) and the downregulation of 257 (3.59%) protein coding genes Many of the upregulated genes in the treated biofilms encode membrane-related and regulatory proteins. In addition, several genes coding for drug resistance determinants (including various efflux systems) were also upregulated. Many genes encoding chemotaxis and motility - related proteins were upregulated, and combined with the down-regulation of an adhesin-encoding gene, this suggests that sessile cells are trying to "escape" from the biofilm. We also observed differential expression of 19 genes coding putative small RNA molecules (sRNA). While the function of these putative sRNAs is at present unclear, their differential expression suggests they may also play a role in chlorhexidine tolerance. A comparison of our data with transcriptomic data obtained in planktonic *Pseudomonas aeruginosa* cells treated with chlorhexidine indicate that there are both common and species-specific mechanisms leading to chlorhexidine tolerance.

Keywords : biofilm, *Burkholderia cepacia* complex, chlorhexidine, disinfection

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Nitric-oxide-gas-releasing solution acting as an antifungal treatment against fungi associated with Tinea Pedis

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Introduction: Most cutaneous infections are the work of the homogeneous group of keratinophilic fungi known as dermatophytes. Tinea Pedis, also known as Athlete's Foot, is a common problem and is probably the most common form of dermatophytosis. All currently available topical therapies require diligent daily applications for a prolonged period of time in order to be efficacious. The dermatophytes *Trichophyton rubrum* and *Trichophyton mentagrophytes* are the major cause of Tinea Pedis.

Nitric oxide is a simple molecule produced in the body which acts as a non-selective broad range antimicrobial which is key to the innate immune system. Recently, it has become increasingly evident that this small gaseous molecule possesses antimicrobial effects on a wide range of microorganisms including bacteria, viruses, fungi and yeast.

In this study we used nitric oxide releasing solution to eradicate fungi associated with clinical Tinea Pedis.

Methods: A novel nitric oxide releasing solution (NORS) was donated (NSI Inc., Canada). Fungi (*T. mentagrophytes* and *T. rubrum*) were grown on plate to mycilial phase (24-48 hrs) or spore stage (5 days). Glass beads were placed in the plate, Shaken for 5 min, transfered media and grown at 30ºC for 24-48hrs. Then 100 µL of the diluted media was put into 3ml NORS at different strengths (A, B). After 30 min at 30ºC samples were plated and incubated. A similar experiment was repeated with beads covered with spores in NORS at 3 different strengths (A, B, C) and at 30 and 60 min at 37ºC.

Results: At mycelia phase – NORS at strength A had a significant reduction on all fungi tested while strength B eradicated all fungi. At spore phase – *T. Mentagrophyte* was eradicated after 30 min at strengths B and C and at all strengths after 60 min. *T. Rubrum* – was not eradicated after 30 min at all strengths but had a significant reduction at strength C and was completely eradicated after 60 min with strength C.

Conclusions: This research suggests that a single 30-60 min exposure to NORS can be used as an effective antifungal agent to treat Tinea Pedis. These are promising results since this NORS may treat fungi both at the mycelia and spore phase with one single dose. Further studies are warranted and may have significant beneficial implications for a rapid single treatment for Athlete's Foot.

Key words: Tinea Pedis, nitric oxide, fungi

Optimizing the sporicidal activity of peracetic acid in food surface sanitation process

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Cleaning and sanitation of processing equipment is a prerequisite to achieve required hygienic standards in food processing. The term sanitation refers to the inactivation of microorganisms through disinfectants, on previously cleaned surfaces and materials. In food industry, peracetic acid may present bactericidal and sporicidal activity at room temperature and is used as sanitizer for food surfaces as well as disinfectant for fruits and vegetables. The aim of this study was to quantify the impact of peracetic acid on endospores of *Bacillus* as a function of environmental conditions.

A psychrotolerant strain belonging to *Bacillus cereus* group, *Bacillus weihenstephanensis KBAB4* was used in this study as a model. Spores were inactivated by peracetic acid solutions (Oxyanios 5, Anios Laboratoires, Lille-Hellemmes, France) of various concentrations, *i.e.* 0,25 g/L; 0,45 g/L; 0,65 g/L; 0,85 g/L and 1,05 g/L after storage at four temperatures 5, 10, 15 and 20°C. The survival kinetics were enumerated using spiral plate count (WASP1, Don Withley Scientific LTD, Shipley, England) on Nutrient Agar (Biokar, Beauvais, France) after acid neutralisation. The survival curves were fitted by the Weibull model (Peleg and Cole, 1998) as presented in Figure 1.

Using an approach adapted from a Bigelow model used in the field of heat treatment (Couvert, 1999), this study proposes to simulate the impact of peracetic acid on spore destruction with the following model : $\log(\delta) = \log(\delta^*) - \frac{T - T^*}{Z_T} - \frac{C - C^*}{Z_c}$ with δ-value, which is the time that leads to a 10-fold reduction of surviving population, at a temperature of storage T and a concentration C of peracetic acid. This δ-value is estimated from a reference value (δ^*), the bacterial destruction at reference temperature (T^*) and concentration (C^*). Z_T and Z_C are, respectively, the variation of temperature from T^* *or* C^*, which lead to a 10-fold reduction δ-value.

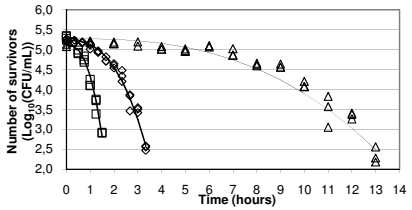

Figure 1 : Examples of inactivation kinetics of *Bacillus weihenstephanensis* KBAB4 by peracetic acid. (□) 0,85 g/L, 20°C ; (◇) 0,45 g/L, 20°C ; (△) 0,45 g/L, 10°C ; (▬) curbs fitted from Weibull model.

This model presents a good quality of fit (RMSE = 0, 04) and offers a user-friendly approach with simple and practical interpretation of its parameters. A log linear decrease of bacterial population for a given concentration of peracetic acid, time and temperature of treatment was successfully quantified by the δ parameter. Spore population was shown to decrease with increasing peracetic acid concentration and increasing storage temperature. Knowing a targeted value of bacterial spore destruction, time of treatment may be estimated in reference conditions (20°C and 1, 25 g/L peracetic acid) or optimised for industrial conditions.

Keywords disinfection; biocide; resistance; modelling; food-born pathogen; Weibull; Bigelow.

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Potential use of silver as an antimicrobial: factors affecting its efficacy

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Despite the widespread use of silver as an antimicrobial agent, there is still much to be learnt about the chemical interactions taking place between the active silver species, the different bacteria and the matrix within they interact. Previous literature on the efficacy of silver based antimicrobial systems reports varied results regarding MIC values, categorical breakpoints and standardization of silver ion biocidal tests. This task is particularly complex, as many solubility issues affecting speciation and bioavailability of silver are still unknown. In the present work, silver prolonged exposure to bacterial contamination as well as to different factors affecting the bioavailability of the active silver species was studied. Growth curves were performed for 2-5 days with the Gram positive *Listeria monocytogenes* and the Gram negative *Salmonella spp.* in Tryptic Soy Broth (TSB) and M9 minimal medium (M9) in the presence of silver ions and silver solutions previously in contact with the growth media. It was found that, after a period where viable counts were not detected, bacterial populations recovered and were able to proliferate in most cases. This could distort the reliability of previously published results on antimicrobial efficacy.

Inactivation of the active silver species was found to be highly influenced by time dependant chemical reactions taking place in the environment of exposure, producing differences of at least 3 orders of magnitude between results for environments with high natural organic matter content and results for aqueous salt buffers. Chloride ions lead to the formation of highly insoluble silver chloride complexes. However, this process can be easily altered by changing the ionic strength or adding new ligands to the solution. Thiol groups like in cysteine strongly bind free silver ions (FSI) producing a strong decrease in antimicrobial efficacy. However, these groups do not seem responsible for the decrease in antibacterial efficacy, as proposed by Glover et al. Methylated sulphur groups like in methionine have far less affinity to FSI. Accordingly, methionine can be supplemented to M9, allowing the tested strains to grow properly without compromising silver antimicrobial efficacy. Under these conditions, an antibacterial effect was found for concentrations as low as 1-10 ppb. Therefore, M9 medium supplemented with Methionine is proposed as a tool to evaluate the full potential of silver-based antibacterial systems.

These results indicate that there is a need for further investigation and understanding of silver as an antimicrobial and about the optimum conditions required for designing silver-based antibacterial systems.

Keywords: antimicrobial silver, silver inactivation, silver speciation, ligands, microbial growth, chlorides, sulfides

Chris N. glover, Sonia K. Sharma, and Chris M. Wood "heterogeneity in physicochemical properties explains differences in silver toxicity amelioration by natural organic matter to daphnia magna" Environmental Toxicology and Chemistry, vol. 24, no. 11, pp. 2941–2947, 2005

Resistance of amoebal cysts and trophozoites to disinfection treatments commonly used in healthcare settings

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In addition to their intrinsic pathogenicity, amoebae are known to potentially harbour pathogenic bacteria that can present a threat to humans and particularly hospitalized patients (2). These bacteria also potentially survive in amoebal cysts, as demonstrated for mycobacteria. It is thus important to evaluate the effectiveness of chemical disinfectants used for surface disinfection against amoebal cysts and trophozoites.

For this purpose we used collection and field isolates of various *Acanthamoeba* species that were encysted using three different protocols (1). Cysts were then diluted in each disinfectant tested (see below), and incubated for various times at appropriate temperature. After neutralization cysts were serial-diluted on *E. coli* lawns on non-nutrient agar to observe re-growth, and log reductions were calculated. Actively growing trophozoites directly taken from culture medium were evaluated using the same method.

We demonstrated that all treatments were active against trophozoites, except glutaraldehyde that presented limited trophocidal activity even after 30 min exposure. Furthermore, we observed high variability in efficacy of biocides against cysts depending on amoebal strains and methods used to produce cysts. The most effective treatments were bleach 2.5% and a peracetic acid-based formulation, with a minimum 4-log10 reduction for each of the 9 strains tested. Ortho-phthalaldehyde (OPA) alone at 0.55%, peracetic acid alone at 0.2% and a hydrogen peroxide-based product (SporKlenz) presented good activity. Bleach 0.25%, a commercial OPA-based product and glutaraldehyde 2% presented a moderate to low activity. Products with the lowest activity were hydrogen peroxide alone at 7.5% and a commercial, 2% glutaraldehyde-based product.

Biocides tested	Contact times	*Acanthamoeba* spp. environmental isolates 1 (river water)	2 (river water)	3 (hospital)	4 (hospital)	5 (river water)	6 (river water)	*A.castellanii* CCAP 1501/10	*A.castellanii* ATCC 30010	*A.polyphaga* CCAP 1501/18
55°C	10min	0.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.5 ± 0.0	0.8 ± 0.1	1.0 ± 0.1	0.5 ± 0.2	0.6 ± 0.1
65°C	10min	> 4.7	> 4.8	> 4.7	> 4.7	> 4.8	> 4.1	> 5.1	> 4.7	> 4.5
Sodium hypochlorite 2.5%	10min	> 4.7	> 4.8	> 4.7	> 4.7	> 4.8	> 4.1	> 5.1	> 4.7	> 4.5
Sodium hypochlorite 0.25%	10min	2.9 ± 0.1	3.5 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	1.3 ± 0.2	> 4.1	> 5.1	2.9 ± 0.2	1.8 ± 0.2
	20min	> 4.7	> 4.8	1.4 ± 0.1	3.6 ± 0.1	> 4.8	/	/	> 4.7	> 4.5
	30min	/	/	2.6 ± 0.2	> 4.7	/	/	/	/	/
Ethanol 70%	10min	> 4.7	> 4.8	2.8 ± 0.1	4.3 ± 0.3	3.5 ± 0.2	> 4.1	> 5.1	> 4.7	> 4.5
Glutaraldehyde 2%	10min	1.6 ± 0.4	0.2 ± 0.2	0.0 ± 0.1	1.1 ± 0.6	4.6 ± 0.1	> 4.1	2.6 ± 0.1	1.5 ± 0.2	1.3 ± 0.3
Glutaraldehyde-based product	10min	1.3 ± 0.2	0.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.8	0.5 ± 0.3	1.6 ± 0.2	2.8 ± 0.2	1.3 ± 0.3	1.2 ± 0.1
	20min	3.9 ± 0.1	0.7 ± 0.1	0.0 ± 0.0	1.9 ± 0.2	2.4 ± 0.1	2.2 ± 0.2	2.8 ± 0.2	2.4 ± 0.1	> 4.5
	30min	> 4.7	0.9 ± 0.1	0.9 ± 0.1	> 4.7	2.5 ± 0.1	2.3 ± 0.2	2.8 ± 0.1	> 4.7	/
Orthophthalaldehyde (OPA) 0.55%	10min	> 4.7	> 4.8	3.7 ± 0.2	1.1 ± 0.3	> 4.8	> 4.1	> 5.1	> 4.7	> 4.5
OPA-based product	10min	2.8 ± 0.2	3.8 ± 0.1	2.6 ± 0.2	1.1 ± 0.1	4.6 ± 0.0	> 4.1	3.0 ± 0.1	2.6 ± 0.1	2.2 ± 0.1
	20min	> 4.7	> 4.8	2.9 ± 0.1	3.7 ± 0.1	/	/	> 5.1	> 4.7	> 4.5
	30min	/	/	> 4.7	> 4.7	/	/	/	/	/
Hydrogen peroxide 7.5%	10min	1.1 ± 0.5	0.3 ± 0.3	1.2 ± 0.1	1.1 ± 0.3	2.7 ± 0.2	1.6 ± 0.2	0.8 ± 0.6	0.4 ± 0.3	2.5 ± 0.4
	20min	3.3 ± 0.1	1.2 ± 0.2	0.5 ± 0.2	1.2 ± 0.2	> 4.8	> 4.1	2.7 ± 0.2	2.2 ± 0.1	> 4.5
	30min	3.8 ± 0.1	1.4 ± 0.2	0.9 ± 0.1	1.7 ± 0.2	/	/	3.1 ± 0.1	2.8 ± 0.2	/
Hydrogen peroxide-based Resert HLD	10min	> 4.7	> 4.8	> 4.7	4.3 ± 0.4	> 4.8	> 4.1	> 5.1	> 4.7	> 4.5
H_2O_2/PAA-based SporKlenz RTU	10min	> 4.7	3.6 ± 0.5	1.6 ± 0.2	1.7 ± 0.8	4.1 ± 0.2	> 4.1	> 5.1	> 4.7	> 4.5
	20min	/	> 4.8	> 4.7	4.1 ± 0.8	/	/	/	/	/
	30min	/	/	/	> 4.5	/	/	/	/	/
Peracetic Acid 0.2%	10min	> 4.7	0.9 ± 0.3	> 4.7	0.2 ± 0.2	> 4.8	> 4.1	> 5.1	2.1 ± 0.5	> 4.5
PAA-based STERIS-20	10min	> 4.7	> 4.8	> 4.7	4.0 ± 0.2	> 4.8	> 4.1	> 5.1	> 4.7	> 4.5

Table 1: Log reductions observed for cysts of 9 *Acanthamoeba* strains treated with various biocides

This study demonstrates that biocides efficacy against amoebae can vary, with known high level disinfectants that may be taken for granted as being effective under clinical practice presenting only limited efficacy. Further investigations are consequently needed to better understand the activity of biocides against amoebal cysts and trophozoites, as well as against bacterial pathogenic species potentially present in amoebae.

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Resistance of selected bacterial species originating from the food industry to different disinfectants

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In this study we have tested the efficacy of disinfection agents against bacterial isolates originating from the food industry (dairy and meat) and obtained from food contact surfaces within 2 hrs after sanitation. Representatives of Gram-positive bacteria (22 isolates of *Staphylococcus aureus* and 65 isolates of *Staphylococcus epidermidis*) and Gram-negative bacteria (30 isolates of *Klebsiella* spp. and 74 isolates of *Escherichia coli*) were included. Disinfectant agents were tested on both planktonic and biofilm cells (75 biofilm-positive isolates). Selected disinfection agents were benzalkonium chloride (BAC), sodium hypochlorite (NaClO), chloramine B (CAB) and peracetic acid (PAA). The disinfectants were tested in tryptone soy broth in two-fold dilution range by using the standard microbroth dilution method. For planktonic cells the minimum inhibitory concentrations (P-MICs) and minimum bactericidal concentrations (P-MBCs) were determined. Similarly, for biofilms the minimum biofilm inhibitory concentrations (B-MICs) and minimal biofilm bactericidal concentrations (B-MBCs) were determined as well. The highest variability in susceptibility was observed with BAC (between the isolates of the same species as well as between those of different species). This was especially noticeable between Gram-positive and Gram-negative bacteria (Gram-positive bacteria were substantially more susceptible). P-MICs of BAC were also lower than P-MBCs and B-MICs. For other disinfectants P-MICs, P-MBCs and B-MICs were very similar within and between different bacterial species. For all disinfectants B-MBCs were significantly higher than B-MICs, P-MICs and P-MBCs. For 50 selected biofilm positive isolates we have determined minimum efficient concentrations (MEC) in water, i.e. those concentrations which reduce a number of viable cells for ≥ 5 log10. To determine minimum efficient concentrations for planktonic cells (P-MECs) end minimum efficient concentrations for biofilms (B-MECs), the suspension test according to the EN 1276:1997 was modified to be applied in microtitrate plates. In contrast to BAC where P-MECs were higher than P-MICs or P-MBCs, in all other disinfectants P-MECs were markedly lower than P-MICs and P-MBCs. Low MEC values of NaClO, CAB and PAA when compared with MIC and MBC values showed on a great instability and low efficacy of these agents in the presence of organic matter. Resistance to disinfectants in water generally increased in biofilms comparing to planktonic cells but this phenomenon was not as obvious as in TSB. A relatively high resistance of bacterial biofilms, when compared with planktonic cells, was clearly observed only with BAC and NaClO. However, unexpected but not unique finding was a relatively low resistance of bacterial biofilms to PAA and especially to CAB. This indicates that biofilms should not always be considered as a highly resistant bacterial form and that a level of their resistance to biocides may greatly be influenced by the presence of organic matter.

Keywords food hygiene; chemical agents; sanitation, resistance, disinfection, milk, food safety

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Review on the efficacy, safety and clinical importance of the antiseptic agent polihexanide (PHMB), a modern and well tolerated alternative to Chlorhexidine

Hübner NO and Kramer A

In the last decade, it has got evident that antimicrobial chemotherapy is limited by the spread of antimicrobial resistance. In parallel, more invasive techniques for monitoring or treating patients and an in average older population of patients in many countries increase the risk of infection.

Therefore, preventive measures to avoid infections have become more and more important. One effective way to avoid the development of new bacterial resistances and to circumvent existing ones is to substitute antibiotics by antiseptics when ever possible. Clinical antisepsis is very effective to prevent transmissions of pathogens as well as for treatment of infections, and is therefore, one of the most promising anti-infective tools today.

While chlorhexidine is still one of the most widely used antiseptic substances worldwide, its effectiveness and safety have been questioned recently. It has been banned for antisepsis of mucous membranes in Japan and is seen more and more critical in other countries due to its cytotoxicity and allergical and toxicological risks.

Fortunately, new, highly effective antiseptic substances with a broad antimicrobial spectrum are available. One of them is polihexanide (polyhexamethylene biguanide, PHMB), a very close but less toxic sibling of chlorhexidine.

This review focuses the different clinical fields of application of PHMB, as special preparations for antisepsis and care of wounds, eye and oral cavity as well as decolonization of MRSA are available and compares the new substance with chlorhexidine and other antimicrobials like silver compounds to show the therapist where it and can add a most welcome alternative to antibiotics or less well tolerated antimicrobial therapies.

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Single Layer Centrifugation with Androcoll™-P as an alternative to antibiotics to control bacterial contaminants in boar semen doses

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Introduction: Most boar ejaculates are contaminated to some extent with bacteria from the environment during semen collection. Antibiotics are added to boar semen extenders used to prepare semen doses for artificial insemination to control the growth of these bacteria, both to prevent disease in inseminated sows and to maintain sperm survival. An alternative bacterial control strategy to adding antibiotics would be to remove the contaminating bacteria from the semen immediately after collection. The objective of the present study was to investigate whether Single Layer Centrifugation (SLC) through Androcoll™-P is able to separate boar spermatozoa from bacterial contaminants in semen. **Methods:** Semen was collected from 10 boars using the gloved-hand method and was immediately extended in warm (35°C) semen extender (modified Beltsville Thawing Solution, i.e. without antibiotics). Aliquots of boar ejaculates (10) were processed by SLC using Androcoll™-P (1). The resulting sperm pellets were harvested in a laminar air flow (LAF) bench and resuspended, with the resulting sperm suspension being aliquotted into separate tubes. The SLC-selected sperm samples and the uncentrifuged sperm samples were subsequently stored in a climate-controlled box at 16-18°C. Aliquots of all sperm samples were submitted for bacterial culture, identification and quantification immediately after preparation (0h) and again after storage for 24h. **Results:** Nine of the ten uncentrifuged samples contained bacteria (total count in the nine samples was 18520 cfu/mL at 0h and 41400 cfu/ml at 24h). The bacteria involved included *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Burkholderia*, *Citrobacter*, *Pantoea*, *Eschericia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. In contrast, five SLC-samples contained no bacteria at 0h or 24h. Four SLC-samples had slight bacterial growth at 0h (total 1270cfu/mL) that in most cases was not increased at 24h (total 940cfu/mL). Bacteria were more likely to be found in the SLC-selected samples if the samples were not processed immediately after collection. One SLC-sample contained bacteria at 0h but not at 24h, suggesting accidental contamination of one of the aliquots after SLC-preparation. Bacteria found after SLC tended to be motile, flagellated bacilli (e.g. *Bacillus* spp). Gram-positive bacteria (e.g. *Staphylococcus* spp.) were found after SLC only if the original sample was heavily contaminated. **Discussion**: It is possible to collect boar semen with only low levels of bacterial contamination by strict attention to hygiene. Bacterial contamination that does occur can be removed, or substantially reduced, by SLC and, furthermore, any bacteria present do not tend to increase in numbers during storage at the normal storage temperature for boar semen (16-18°C) for 24h. In contrast, there was a marked increase in bacterial numbers in the uncentrifuged samples during storage, despite the storage temperature being lower than optimal for bacterial growth. Thus incorporation of SLC in boar semen processing could enable antibiotic usage in boar semen extenders to be reduced. However, neither SLC-processing, nor the use of antibiotics, is a substitute for good hygiene practices on boar studs. Further studies are required to investigate whether the same results can be obtained without the use of a LAF bench, since such equipment is not usually found on semen collection units.

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Keywords: Androcoll™-P, Single Layer Centrifugation, boar semen, alternatives to antibiotics

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Sodium Tripolyphosphate Anti-*Candida* activity

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Introduction:

Sodium Tripolyphosphate (STPP), also known as Pentasodium triphosphate or Pentasodium Tripolyphosphate, is a polyanion classified by the Food and Drug Administration as a Generally Recognized Safe Substance (GRAS). It is approved as a food additive either in USA and in Europe, and has been used particularly in cured meat and seafood on behalf of its ability to improve flavor, water retention and decrease cooking shrinkage [1]. In the context of pharmaceutical technology, STPP has been applied to formulate chitosan nano and microparticles due to its ability to interact with the chitosan free amino groups by electrostatic forces. Because of their highly charged anionic nature, polyphosphates have the capacity to quelate cations. It has been suggested that this ability to form stable complexes with cations, turning them unavailable for metabolic functions, causes the microorganisms growth inhibition [1]. In fact, studies on the antimicrobial activity of STPP, along with other polyphosphates, over food contaminants have been conducted particularly during the 80 and 90 decades suggesting their possible use as conservatives [1, 2]. However studies on STPP *in vitro* antimicrobial activity against clinical isolates are very scarce [3]. Vulvovaginal candidosis is one of the most common clinical presentations of *Candida spp infections*, affecting 70-75% of women at least once in their lifetime and with a high rate of recurrence [4]. *C. albicans*, followed by *C. glabrata*, *C. tropicalis* and *C. krusei* are the main species responsible for this infection. Difficulties in the clinical management of this infection, particularly in recurrent forms, are impelling scientists to search for new therapeutic alternatives. In this study, the *in vitro* anti*Candida* activity of STPP was evaluated.

Methods:

Sixteen clinical and collection-type *Candida* strains were studied including *C. albicans* (1 ATCC, 4 clinical strains); *C. glabrata* (5 clinical strains); *C. tropicalis* (5 clinical strains) and *C. guilliermondi* (1 clinical strain). A 5% STPP solution was prepared in sterile water followed by filtration through a 0.22 µm filter. Tests were performed according to CLSI reference M27-A3 protocol. Minimal inhibitory concentrations (MIC) were determined after 48 hours of incubation at 37ºC. Yeast growth was visually compared for each concentration with the control sample. All determinations were performed in duplicate for each assay. Assays were repeated three times.

Results:

STPP was active against all tested strains but it was particularly active against *C.glabrata*. For *C. albicans, C.tropicalis* and *C.guilliermondi* MIC varied from 6,25mg/mL to 12,5 mg/mL while for *C. glabrata* it was as low as 0,391mg/mL. MLC values differed from MIC values and were similar for all tested strains (25-50 mg/mL).

Conclusions:

STPP was shown to have antifungal activity against all tested *Candida* strains and to be particularly active against *C. glabrata.* The differences between MIC and MLC values show that at low concentrations the product exhibits fungistatic activity but at higher concentrations it becomes fungicidal. Therefore STPP inclusion in vaginal drug delivery systems may contribute to an overall antifungal activity in the treatment of vulvovaginal candidosis.

Keywords Vulvovaginal candidosis; STPP; polyphosphates; Candida spp.

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Susceptibility to Benzalkonium Chloride in a Collection of Clinical S. *Aureus* Isolates

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Quaternary ammonium compounds are largely used as both household and hospital settings disinfectants; particularly in hospital, correct disinfection practices are the first line of defence against nosocomial-acquired infections. However, the mode of action of biocides should be thoroughly known, in order to formulate and apply the appropriate disinfection protocols and policy guidelines. *S.aureus*, as well as other microorganisms of clinical importance, are known for their ability to survive on inanimate surfaces, thus raising concern on whether the ability of a given biocide to kill a microorganism in active growth (as is usually tested) may represent a faithful picture of what may happen to germs growing in a biofilm.

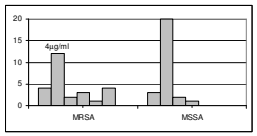
We evaluated the MIC of benzalkonium chloride (BKC) in a collection of *S.aureus* clinical isolates, either methicillin susceptible (MSSA) or resistant (MRSA). We wanted to determine what kind of distribution was present in a population from a clinical setting as far as the resistance to one of the most commonly used biocide was concerned. Moreover we wish to evaluate whether a correlation existed between the resistance to BKC, methicillin resistance and the ability to form biofilm.

MIC to BKC and ability to form biofilm was determined for twenty-six MRSA and 26 MSSA isolates from various types of invasive infection (sepsis, necrotizing pneumonia, abscess, etc); *S.aureus* ATCC6538, the reference strain used for efficacy evaluation of disinfectants by the European norms, was used as control.

Considering the collection on the whole, the majority of *S.aureus* isolates (30/52) had a MIC = 4µg/ml. However, when grouping MRSA and MSSA, almost all MSSA (20/26) had a MIC = 4µg/ml; 3 isolates had a MIC of 2µg/ml and 3 up to 8µg/ml. Concerning the MRSA group, also in this case almost 50% of the strains had a MIC of 4µg/ml; however, there was also a small cluster (4 isolates) with a MIC=16 µg/ml and, in general, they appeared less homogeneous compared to MSSA.

In the figure, bars represent the number of strains growing at different BKC concentration (from 2-4 µg/ml to 16 µg/ml, bars from left to right).

MIC of the reference strain, *S.aureus* ATCC6538 was in line with that of the majority of strains (4 µg/ml).



As expected, MRSA produced significantly thicker biofilm compared to MSSA (median OD570nm 0,350 and 0,280 and 1,233 vs. 0,900, with and without glucose, respectively – $p<0,02$), although no direct correlation could be found between, for example, higher biofilm OD and higher MIC.
To determine whether the ability to form biofilm had any role in the increased MIC, a short-term MBC (the lowest concentration of BKC able to kill bacteria in the shortest time) was determined for the reference strain: a concentration of 250µg/ml of BKC was necessary to kill bacteria after 1 hour contact. A range of concentrations were then tested on 24 hrs-mature biofilm and on planktonic cells. Surprisingly, instead of biofilm-embedded cells being more resistant compared to planktonic ones, a BKC concentration of 0,125 µg/ml was sufficient to abolish any re-growth from biofilm.
Preliminary experiments indicate that the biofilm carbohydrates may support the enhanced efficacy of BKC against *S.aureus* biofilm-growing cells.
Further studies are underway to characterize the MRSA showing the highest MIC and to gain insights into the enhanced efficacy of BKC against biofilm-embedded cells.

Keywords S.aureus, Biocides, Biofilm, Resistance

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Thermodynamic of bacterial adhesion to stainless steel surfaces conditioned with silver nanoparticles

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Use of silver nanoparticles, due its antimicrobial potential, has emerged as a strategy for inhibiting microbial adhesion, a phenomenon that occurs naturally in aqueous medium and depends on the interfacial properties of microorganisms and substrates/surfaces. The hydrophobicity of surfaces plays an important role in the microbial adhesion process. The adherence of two surfaces in aqueous medium is facilitated if the water is removed between the surfaces. The hydrophobicity of the surfaces contributes to the water removal. If the two surfaces are hydrophobic, it is easier to eliminate the water layer. In this context, this research aimed to evaluate the ability of dispersion of silver nanoparticles to modify the thermodynamic characteristics of stainless steel surface in order to reduce the process of adhesion of *Pseudomonas aeruginosa, Listeria innocua, Staphylococcus aureus and Escherichia coli.* It was found that the dispersion of silver nanoparticles were able to decrease the contact angle of stainless steel when conditioned with water of 73.20° to 12.10° when conditioned with dispersion of silver nanoparticles, making this surface more hydrophilic. The total free energy of interaction was 41.46 mJ/m^2 and -46.84 mJ/m^2 for the surfaces conditioned with the dispersion of silver nanoparticles and water, respectively. Thermodynamic of adhesion for all the bacteria tested was less favorable when the stainless steel surfaces were conditioned with the nanoparticles. Nanoparticles of silver, therefore, have antimicrobial efficiency and besides to alter surface hydrophobicity which is important for the cell adhesion, also present a large antimicrobial spectrum.

Keywords: dispersion of silver nanoparticles; hydrophobicity; stainless steel; antimicrobial

3. Evaluation. Clinical and pre-clinical trials

A comparative evaluation of combination therapy of fluconazole 1% and urea 40% compared with fluconazole 1% alone in a nail lacquer for treatment of onychomycosis: therapeutic trial

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Objective
Onychomycosis is a common disease affecting as much as 8% of the general population. The incidence of this infection is increasing word wide. Treatment of onychomycosis is challenging, complicated by low cure rates and relatively high relapse rates. The purpose of this study is to assess the safety and efficacy of combination therapy of fluconazole 1% and urea 40% compare with fluconazole 1% alone in a nail lacquer for treatment of onychomycosis caused by dermatophytes.

Material and Methods
This is a randomized, double-blind, study enrolling 66 patients with onychomycosis of the fingernails and toenails. Clinical and mycological efficacy as well as measures of safety was assessed monthly for a maximum of 6 months of treatment. We choose a total of 66 patients with severe toe and finger nails onychomycosis due to dermatophytes. The etiologic agents of onychomycosis were established by microscopic examination by potassium hydroxide solution (20%) and culture. The treatment regimens were: fluconazole 1% once daily for 6 months in 16 cases, and fluconazole 1% with urea 40% for 6 months in 16 cases.

Results
The high rates of clinical success achieved at the end of therapy in patients treated with fluconazole 1% with urea 40% about 64% and fluconazole 1% alone51%. Results of the treatment were followed up for 24 weeks. Thinning and total avulsion of the nails occurred in 87% of 32 patients with Dermatophytes onychomycosis and regrowth of a normal appearing nail was found in 82% of 48 patients. Distal and lateral subungual onychomycosis was the most type of onychomycosis in this study (79.2%). Failure rate was 22.6% and recurrence rate was 12% in patients. Results of the treatment were followed up for 24 weeks. In this study topical treatment of onychomycosis with a combination of 1% fluconazole and 40% urea, was more effective than 1% fluconazole nail lacquer alone. Side effects were negligible.

Conclusion
Topical antifungals can possibly better penetrate the thin nail plate. Fluconazole therapy is effective and safe alternative in treatment of onychomycosis due to Dermatophytes. Our study shows that treatment with fluconazole 1% and urea 40% (once daily for 6 month) is cost effective and safe in treating onychomycosis due to dermatophytes.

Key words: Onychomycosis, topical therapy, fluconazole

A first prospective randomized controlled trial to decrease bacterial load on chronic wounds in patients using a cold atmospheric plasma device

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We are treating chronic wounds using a low-temperature argon plasma. This is the first clinical study to show a highly significant decrease in the rate of germ load in plasma-treated wounds (34%, $p<10^{-6}$) in patients in comparison with wounds that received only standard wound care. The reduction is found in all kinds of germs, regardless the resistance level and the treatment is very well tolerated and no side effects occurred until now. The results of this study revealed the potential of atmospheric argon plasma treatment as a new approach to kill bacteria.

The observed bactericidal effect of plasma therapy relies on the synergy of UVR, charged particles, electric fields, ROS and RNS. The combination of these biologically active components makes plasma a promising tool for fighting multiresistant germs. The advantage of this indirect plasma device is that it can be designed and optimized for different purposes, such as germ specific biofilms or varying wound fluid compositions.

Furthermore there is evidence that plasma can enhance wound healing itself.

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Adherence to ART and its associated factors among HIV Aids Patients in Addis Ababa

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Background: Customary practice; fasting and holly water use is very common in Ethiopia, and most people belong to one of religious denomination, as a result the majority of them share common spiritual believes. It is recommended that for any ARV program there should be a concurrent plan for adherence assessment and support, this study was undertaken to investigate the influence of the socio-cultural factors among HIV AIDS patients in Addis Ababa.

Objective: The main objective of the study was to determine adherence levels and identify demographic and socio-cultural factors that influence Adherence to ART among HIV AIDS Patients.

Methods: This study was conducted in ten health centers of Addis Ababa which are providing ART service from January, 2010 to April, 2010. A cross sectional survey design was used to conduct the study. Data was collected through patient exit interviews of 872 PLHIV who were on ART. Data analysis was conducted in SPSS V.16. Data was checked for normality and descriptive statistics was generated for quantitative data. Binary logistic regression tests were performed to analyze the degree of association between the dependent and the independent variables.

Result: A total of 872 patients were interviewed. Self reported dose adherence in the study area was 87.7%. Customary practices; fasting, religious place attendance and spiritual believe were used to measure level of spirituality. High spirituality was found to have significant association **2.781 [1.230 – 6.287]** $P< 0.05$ with non-adherence. Forgetting, fear of stigma or disclosure, and side effects were identified as the major reasons for missing ARV. Demographic variables were not found to have significant association with the level of adherence. The study reveals that customary fasting practice pose problem which may cause sub optimal adherence.

Conclusion and Recommendation: High spirituality was found to be the main challenges for appropriate adherence. Therefore, strengthen the existing adherence-counseling strategy, involving religious leaders in designing and implementing continues patient education tools and operational research are recommended.

Key words: - HIV/AIDS, adherence, antiretroviral therapy

Clinical Manifestation of Bacteremia Caused by *Stenotrophomonas maltophilia* in Children for a Ten-year Period: A Single Center Study

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Background: Severe infections caused by *Stenotrophomonas maltophilia* are associated with high mortality in children. The successful treatment for *S. maltophilia* bacteremia in children poses further challenges for pediatricians due to limited choice for medication; trimethoprim/ sulfamethoxazole (TMP/SMX) is not recommended for infants under 2 months old and quinolones are not generally recommended in pediatric population.

Methods: A retrospective chart review was performed for pediatric *S. maltophilia* bacteremia cases at Samsung Medical Center, Seoul, Republic of Korea, from January 2000 to July 2010.

Results: Seventeen children with *S. maltophilia* bacteremia were identified over 10-year period (20 episodes and 37 isolates). The median age was 6.5 yrs (range, 7 days~17.4 yrs) and male to female ratio was 13:4. Eleven (64.7%) were cancer patients including five hematopoietic stem cell transplant recipients. Three (17.6%) were less than two months old who were in the neonatal intensive care unit (NICU), including 2 premature babies. The isolates were susceptible to ceftazidime (7/17, 41.2%), ticarcillin/clavulanic acid (8/14, 57.1%), TMP/SMX (15/17, 88.2%), and levofloxacin (14/14, 100%). The overall mortality rate was 70.6%. The proportion of cancer patients was higher in mortality group (10/12) compared to survival group (1/5) (83.3% vs. 20%, $P<0.05$). The median initial absolute neutrophil count at onset of bacteremia was significantly lower in mortality group than that of survival group (14/μL vs. 5,924/μL, $P<0.05$). Among three infants younger than 2 months old, two patients died.

Conclusion: The treatment of *S. maltophilia* infection presents a therapeutic challenge. Patients having cancer or receiving NICU care are at high risks for acquisition of infection and increased mortality.

Keywords *Stenotrophomonas maltophilia*; bacteremia; children

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Colistin: an effective alternative against multi-drug resistant *Acinetobacter baumannii*

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Introduction: Acinetobacter has emerged as a significant nosocomial pathogen, especially in intensive care units. Increasing incidence of the strains resistant to major groups of antibiotics like carbapenems, glycylcyclines, aminoglycosides and fluoroquinolones have limited the treatment options. Colistin, an older antibiotic, remains sometime only option in the treatment of *A.baumannii* infections.

Objective: To determine the in vitro efficacy of colistin against MDR *A.baumannii* isolated from a tertiary care hospital of Pakistan

Place and duration of study: The study was carried out from September 2009 to February 2010, at the Department of Microbiology, Army Medical College/ National University of Sciences and Technology, Rawalpindi, Pakistan.

Materials and method: Clinical specimens were received from intensive care units and various clinical wards of an 1100 bedded tertiary care hospital of Rawalpindi, Pakistan. Specimens were inoculated on appropriate culture media and incubated at 37^{o}C for 24 hours. *Acinetobacter species* were identified by using standard microbiological procedures (Gram's stain appearance, colonial morphology, catalase test, cytochrome oxidase reaction, motility and by using biochemical tests). Identification up to the species level was done by using Analytical Profile index API 20NE (Biomerieux). Susceptibilities of imipenem, meropenem, ciprofloxacin, gentamicin, amikacin and tobramycin were determined by Kirby-Bauer disc diffusion technique. MDR was defined as absence of susceptibility to aminoglycosides, carbapenems and fluoroquinolones. Minimum inhibitory concentration (MIC) of colistin was performed by using E-strips (AB BioDisk) for each isolate. The MIC results were interpreted according to criteria set by Clinical and Laboratory Standards Institute (CLSI).

Results: A total of 68 MDR *A.baumannii* were isolated during the study period. Colistin exhibited excellent activity against the isolates. All the MDR *A.baumannii* were susceptible to colistin (MIC: ≤2 μg/ml sensitive, ≥4 μg/ml resistant).

Conclusion: MDR *A.baumannii* associated infections are difficult to treat and colistin provides an effective treatment option against this resistant pathogen.

Keywords: Colistin, MDR *A.baumannii*

Effectiveness and Safety of Miconazole with Hydrocortisone (Daktacort) Feminine Care Cream in the Treatment of Vulvar Candidiasis

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Background: About 40-75% of sexually active women experience vulvovaginal candidiasis (VVC) symptoms. Chronic VVC ranks 3rd (10%) among the most common causes of chronic vulvar symptoms referred to a dermatologist. Though not life-threatening, vulvar candidiasis causes genital pruritus, reduced sexual pleasure, and psychological distress leading to impaired quality of life. Current medical literature show that topical antifungal creams with its prompt relief of symptoms, may be used alone or as an adjunct to oral and intravaginal antifungals in the treatment of VVC. This is a Phase IV study to determine the **effectiveness and safety of miconazole with hydrocortisone (Daktacort) feminine care cream in the treatment of vulvar candidiasis.** Study results will help guide clinicians and consumers on the correct usage of this "over-the-counter" drug.

Methods: This was a multicenter, open-label, single arm, prospective clinical trial conducted in Quirino Memorial Medical Center and Dr. Jose Fabella Memorial Hospital (Philippines). After computing for sample size based on 80% power, alpha of 0.05, assumed 80% cure rate, 10-point margin of error and adjusting for 50% drop-out rate, the total sample size was 114 subjects. Statistical analysis was done on intent to treat principle. Descriptive and inferential analyses were performed. All statistical tests were performed at alpha of 0.05.

After obtaining informed consent, non-pregnant and non-lactating females, 18 to 60 years old who passed screening based on inclusion/exclusion criteria, were enrolled. Baseline past history and demographic data were taken. After initial application of study drug, time for pruritus relief was noted using a visual analog scale (VAS). Each subject applied the study drug topically twice daily until follow up after 14 days, or after 28 days if not yet cured. Documentation of the following were done on day of enrollment, and Days 14 and 28: pruritus frequency, extent, intensity and effects on the patient's mood, sleep patterns, sexual desire and function to determine the Modified Itch Severity Scale score. Study is completed on day 14 (± 3 days) if the patient is already cured or up to day 28 (± 3 days). Adverse events were noted. Wilcoxon Signed Ranks test was used for the Modified Itch Severity Scale score. Cure rates and proportion of patients with resolved pruritus was computed. Paired T-test was done to determine the change in VAS pruritus assessment.

Results: A total of 115 patients were enrolled in the study. Seventy-two subjects (63%) followed up until Day 14 and seven of these followed-up until Day 28. There were 43 (37%) drop-outs. Data of those who did not follow-up on day 14 was used for time to pruritus relief but not for cure rate. The cure rate was 88% to 97%. Pruritus relief was achieved in 110 subjects (96%) within 1 hour. Time to itch relief was as fast as 3 minutes in 5% of subjects, 9 minutes in 57% of subjects and mean time to relief was 13.4 minutes. Times achieved for pruritus relief in the drop-out group were similar. There was a highly significant decrease in pruritus frequency, extent and intensity over time. There was also a significant decrease in mood changes, and improvement in sleep, sexual desire and function and a highly significant decrease in the Modified Itch Severity Scale score. Based on this, quality of life was improved due to treatment with miconazole + hydrocortisone (Daktacort) feminine care cream. Two subjects (2%) had adverse events (pruritus), with no serious adverse effects.

Conclusion: Miconazole + hydrocortisone (Daktacort) feminine care cream is effective and safe in curing vulvar candidiasis with rapid resolution of vulvar itch, leading to improved quality of life.

Keywords: miconazole, hydrocortisone, vulvar candidiasis, vulvovaginal candidiasis, Daktacort feminine care cream

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Efficacy of liposome encapsulated ciprofloxacin against *Francisella tularensis* LVS strain

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Liposome encapsulated ciprofloxacin for inhalation (CFI) was investigated as a putative post exposure therapeutic agent against *Francisella tularensis*. *In vitro*, CFI reduced viable intracellular *F. tularensis* live vaccine strain (LVS) numbers to a greater extent than ciprofloxacin. Additionally, a *Galleria mellonella* model of infection with LVS was used to demonstrate that CFI provides protection against a lethal *F. tularensis* LVS infection at lower concentrations than ciprofloxacin. In murine studies, where antibiotics were delivered by intranasal instillation, CFI provided a higher level of protection than ciprofloxacin against a lethal LVS challenge. Consequently, inhaled CFI may be a promising medical countermeasure for use in the event of a deliberate release of biological warfare agents.

Gemcitabine : a "new" antibiotic to fight against resistant Gram positive bacteria

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About 50 000 patients die each year from a *Staphylococcus aureus* disease in spite of adapted microbial drug administration. More and more bacteria have developed one or many resistances against microbial drug, and thus several antibiotics have no effect on bacteria. Consequently, clinicians have to search new molecules in order to prevent bacteria growth.

We evaluated the inhibitory activity of Gemcitabine on different bacteria including some strains resistant towards antibiotics. We also studied the mechanism of resistance.

Gemcitabine is active towards several Gram positive bacteria as *S. aureus* strains and *Enterococcus* strains. Especially Meticillin Resistant *Staphylococcus aureus* (MRSA) and Vancomycin Intermediary *Staphylococcus aureus* (VISA) are sensible to this molecule, with minimum inhibitory concentration respectively from 0.031 µM to 8 µM and from 0.063 µM to 0.5 µM.

Moreover, resistance frequencies are about 10^6 in Meticillin Sensitive *S. aureus (*MSSA) which is similar to many classical antibiotics. Acquisition of resistance is concentration dependant: the more the concentration of Gemcitabine increase, the less resistant strains appear. Mutations in genes of deoxyribonucleotide kinases (*SadAK, SadGK* and *SaTK*), which are involved in Gemcitabine metabolism, seem to be related to these resistances, but are most likely not the only mechanisms.

According to our encouraging results, we propose the use of molecule derived from nucleoside analogues in the fight against bad multiresistant bugs.

Keywords: Gemcitabine, Antimicrobial drug, Resistant strains, desoxyribonucleotide kinases

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In vitro evaluation of clinical relevant parameters of antimicrobial peptides derived from human lactoferrin against Gram-positive bacteria

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Using a short stretch of 11 amino acids rich in positively charged residues from the N-terminal part of human lactoferrin as template, a panel of 173 peptides each possessing a specific chemical structure was synthesized and their antimicrobial activity was characterized. Peptides showed high LPS-binding activity *in vitro* and *in vivo* as well as antimicrobial activity against a broad range of Gram-positive and -negative bacteria. The present study analyzes the action of two peptides composed of 8 and 9 amino acids, respectively, and their N-acylated derivatives (lipopeptides) against Gram-positive pathogens such as *S. aureus*, a leading cause of human bacterial infections worldwide.

For this purpose, we used the peptides and lipopeptides to determine (i) their antimicrobial activity on Gram-positive bacteria by the micro-dilution method, (ii) their kinetics of killing on a MRSA strain by lethality curves assays, (iii) their potential cytotoxicity on fibroblasts, (iv) their antimicrobial activity against *S. aureus* biofilm grown in microplates (v) the emergence of mutants being resistant to the peptides by monitoring bacterial susceptibility after repeated sub-culturing in the presence of sub-inhibitory concentration of the peptides.

The peptides showed an antimicrobial activity against *S. aureus* strains (MIC 4 - 16 µg/ml) and rapid killing kinetics similar to daptomycin. The mutation rate was lower than other clinical antibiotics tested (e.g. ciprofloxacin and erythromycin), whereas the MIC of those antibiotics increased through all the subcultures (8 and 64 times), the pressure of the peptides did not increase the MIC of the strain. Negligible cytotoxicity was observed for the peptides on fibroblasts as detected by the uptake of propidium iodide, which can only penetrate into cells that exhibit cell membrane damage. In this assay melittin was used as a control for 100% cell damage.

Our results clearly indicate that the synthetic peptides derived from lactoferrin are promising antimicrobial agents. Since they are active on clinically relevant Gram-positive as well as Gram-negative pathogens and additionally were shown to neutralize LPS-induced endotoxic shock in animal models, further studies should be done to study the potencial application against polimicrobial infections.

Keywords: cationic peptides, antimicrobial agents, antibiotic resistance, *S. aureus*.

Acknowledgement: ANEPID

Monitoring aminoglycosides in an Intensive Care Unit

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Objectives. – This monocentric, observational and retrospective survey was performed to check the appropriateness between aminoglycoside prescriptions and inhibitor quotient to be reached, in Intensive Care Unit (ICU) patients. We identified variability factors for aminoglycoside plasmatic concentrations at peak such as standardized index of gravity (IGS2 scale), age, sex, weight, and severity of sepsis.

Materials and method. – Eighty-seven ICU patients received an antibiotic combination mandatorily including an aminoglycoside (amikacin or gentamicin) as curative treatment for a severe infection. Prescribed dosages were 15 mg/kg for amikacin and 5 mg/kg for gentamicin. The maximal concentration (Cmax) and minimal inhibiting concentration (MIC) of involved bacteria were recorded. The aminoglycoside ratio Cmax/MIC, called inhibitor quotient, was determined. The inhibitor quotient was considered efficient when superior to 10. The Cmax for aminoglycoside first peak was also compared with the theoretical Cmax to be reached

Results. – In the aminoglycoside Cmax, 50.3% were efficient (59.6% for amikacin Cmax and 38.9% for gentamicin Cmax). In 46% of the cases, the inhibitor quotient was efficient; 12.6% of Cmax reached the theoretical Cmax. Factors identified as negatively interacting with biological efficiency were: Gram-positive bacteria or anaerobic bacteria infections and planned surgery.

Conclusion. – In the inhibitor quotients, 49.7% were at inefficient rates, even when the recommended aminoglycoside dosage for was given. Therefore, dose and administration should be updated.

Keywords*:* Aminoglycosides; Toxicity; Therapeutic drug monitoring; Intensive Care Unit

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Susceptibility of *Aspergillus* spp., *Candida* spp., *Cryptococcus* spp. and *Trichosporon* spp. to Branched Histidine and Lysine Rich Peptides (BHKPs)

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Background The treatment of fungal infections in immuno-compromised patients remains a challenge to clinicians. Serious side-effects and increasing resistance interfere with the success of antifungal therapy. Hence there is an ongoing need for new antifungal agents. A group of branched histidine and lysine-rich peptides (BHKPs) was developed, with a mode of action similar to histatins. The aim of this study was to determine the antifungal spectrum of three BHKPs.

Methods Minimal inhibitory concentrations (MICs) were determined for 9 *Aspergillus* isolates, 5 *Candida* isolates, 21 *Cryptococcus* isolates and 8 *Trichosporon* isolates, according to the CLSI broth dilution methods for filamentous fungi and yeasts. MICs were assessed in triplicate for Amphotericin B (AMB), Voriconazole (VOR), Caspofungin (CAS), Histatin-5 (HST-5) and three BHKPs HK231, HK233 and HK234.

Results MICs of AMB, VOR and CAS obtained for all isolates were similar to MICs described in the literature. All isolates were resistant to HST-5 (MICs >16 µg/ml). The *Cryptococcus* isolates seemed to be susceptible to the three BHKPs, with median MICs of 4 µg/ml (HK231 and HK233) and 8 µg/ml (HK234). The *Trichosporon* isolates seemed also susceptible to HK231 and to HK233 (MICs 8 µg/ml and 16 µg/ml). MICs for HK234 were 128 µg/ml. However, none of the *Aspergillus* isolates and *Candida* isolates were susceptible to the three BHKPs tested, with median MICs of >128 µg/ml.

Conclusion Whereas *Aspergillus* spp. and *Candida* spp. are not susceptible to BHKPs, the basidiomycetous fungi *Cryptococcus* spp. and *Trichosporon* spp. are. Of the three BHKPs tested, HK231 showed highest antifungal potential. Studies in models of fungal infection are needed to determine the *in vivo* activity of these peptides.

Keywords: antifungal susceptibility, peptides

The Effect of Care Bundle Development on Surgical Site Infection Following Hemiarthroplasty: An Eight Year Review.

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Proximal femoral fracture (PFF) is the most common reason for emergency orthopaedic admission in the UK with an annual cost of £1.7 billion to the National Health Service (NHS). Surgical site infection following PFF increases patient mortality and morbidity. *Methicillin Resistant Staphylococcus Aureus* (MRSA) poses a particular risk in this patient cohort as a large proportion of these patients are residents of long term care facilities and are therefore transient or chronic carriers of MRSA. The numbers of Gentamicin-susceptible MRSA strains are increasing and the indiscriminate use of Vancomycin is associated with the development of Vancomycin-resistant bacteria. As well as microbial resistance, *C. Difficile* overgrowth is another significant complication associated with antibiotic prophylaxis. We recorded the effect of three stages of care bundle development on the infection and specifically the MRSA rate following hemiarthroplasty over an eight year period at the University Hospital of North Staffordshire. Data was collated from the Surgical Site Infection Surveillance Service. This data is prospectively collected, independently collated and published on a quarterly basis. Between October 2001 and June 2009, 1830 hemiarthoplasties were carried out. A statistically significant difference in infection rate and MRSA rate between the three care bundles was found following analysis using the Chi-squared test (p value = <0.05). The most effective care bundle included double skin prep using alcoholic Chlorhexidine, a single dose of intravenous Augmentin and Gentamicin at induction and implanted Gentafleece® at wound closure. This care bundle is tailored to prevent MRSA infection and minimise risks associated with antibiotic prophylaxis. The care bundle we propose is a simple and cost effective improvement in the clinical care of this vulnerable group.

Keywords: MRSA, C. Difficile, Surgical site infection, Hemiarthroplasty

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Therapeutic Drug Monitoring of Antimicrobials for Dose Adjustment in Patients from the Intensive Care Burn Unit

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A complex pharmacotherapy approach for the main antimicrobial agents is required for the control of infections in the intensive care burn unit (ICBU) for burn patients with sepsis. Daily prescription for these agents must be considered as a critical decision of the physician to guarantee drug efficacy. A serial of agents like vancomycin, imipenem, cefepin, sulphamethoxazole, piperacillin, oxacillin, ciprofloxacin are required for the treatment. Also, fluconazol is currently prescribed to patients with documented fungal infection due to the long term period in the ICBU associated to immunosuppressant profile of these patients. The aim of the study was to evaluate antimicrobial therapy considering the dose regimen recommended by the committee of control of hospital infection against the dose adjustment required for burn patients based on therapeutic drug monitoring (TDM) and investigation of the pharmacokinetics (PK). Thirty two patients from the ICBU/Plastic Surgery and Burns Division were included in a prospective study, and the protocol was approved by the Ethics Committee of the Clinicas Hospital, Medical School, University of Sao Paulo. Patients of both genders (23M/9F) presented: 39.6+/-17.0 yrs, 69.5+/-9.5 kg (BW), and 33.9+/-20.2 % (TBSA); incidence of thermal injury was 27/32 (84%) against 5/32 (16%) of electrical injuries; also inhalation injury was verified in 11/32 (34%) of those patients. During the follow up, burn patients presented normal renal function and a few ones renal failure in some periods in the ICBU. Pharmacotherapeutic follow up was performed by blood collection samples, 1mL each from the venous catheter into sodium EDTA tubes (BD); samples were centrifuged at 2800g for 30 min and the plasma samples were kept in a deep freezer until assay. During the pharmacotherapeutic follow up of patients in the ICBU, approximately 1500 measurements were performed for TDM purpose and dose adjustment by PK. Exceptionally for patients with renal failure, an additional sample blood collection was performed before the optimization of drug therapy based on the trough. As data published by the same authors, antimicrobial plasma levels were measured by high performance liquid chromatography (HPLC) using two bioanalytical methods reported to determine simultaneously vancomycin, imipenem, cefepime in a profile LC1, and sulphamethoxazole, piperacillin, oxacillin, ciprofloxacin in a profile LC2. Also, for the antifungal therapy, fluconazol plasma level was determined in 12/32 of patients under long term antimicrobial therapy. Plasma curve decay was plotted for each drug considered and pharmacokinetics was analyzed by PK Solutions 2.0 software (Summit, USA). Data obtained suggest that the pharmacokinetics was extensively altered for vancomycin (88%), cefepime (64%), sulphamethoxazole (52%) and also for fluconazole (74%) and in a lower extension for imipenem (19%). Therefore, dose adjustment was required in almost 80% of burn patients treated with these agents. On the other hand, for the other antimicrobials prescribed, the minimum effective plasma concentration (MEC) was reached by empirical dose regimen's prescription. Higher plasma clearance and trough lower than the MEC in patients with severe burns may increase the risk of suboptimal bactericidal action and the development of resistance highlighting the need for dosage individualization. A powerful tool for the optimization of antimicrobial therapy involves TDM plus PK; consequently, it is strongly recommended their application in these situations. In conclusion, the dose regimen must be altered considering the kinetic parameters and also the trough against the MEC for each antimicrobial agent. If the plasma clearance and volume of distribution increase in the same extension, the biological half life remains unchanged; consequently if the trough was lower than the MEC, the total daily dose must be increased remaining unaltered the time dose interval of the new dose regimen. On the other hand, if the plasma clearance reduces but the volume of distribution increases, the time dose interval of the new dose regimen must be predicted based on the biological half life estimated and also the value for the trough.

Keywords: antimicrobials, HPLC, plasma monitoring, pharmacokinetics, dose adjustment

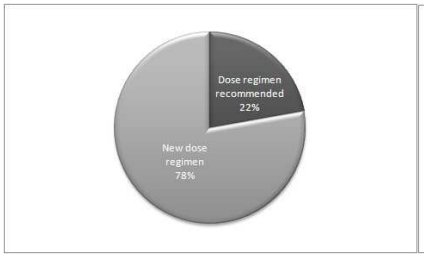

Fig.1. New dose regimen against recommended dose

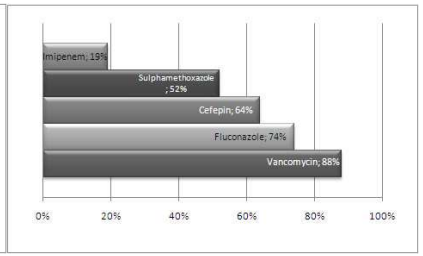

Fig. 2. Dose adjustment required to burn patients

4. Natural products II: Terrestrial and Marine organisms

A cinchona alkaloid derivative as lead compound against *Staphylococcus aureus* biofilms infection

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Traditionally, there has been an intrinsic higher failures rates of antibacterial screenings in drug discovery, when compared to other therapeutic areas, and this failure rate has significantly raised when searching for antibiotics that can be effective to treat pathologies caused by bacterial biofilms. A biofilm is defined as a sessile microbial community attached to a solid surface and embedded in a matrix consisting of extracellular polysaccharides, proteins, water and extracellular DNA. Cells in a biofilm exhibit altered growth rates, reduced metabolic activity and increased chemoresistance towards antimicrobial therapy when compared to their planktonic counterparts. To add more complexity, there is still no standard screening method available for assesing anti-biofilms activity. These features explain why biofilms are one of the most challenging targets in current drug discovery, representing the cause of up to 65% of all infections in developed countries (Hall-Stoodley and Stoodley 2009).

Over the past few years, our group has focused on developing appropriated screening platforms with the ultimate goal of identifying potent anti-biofilms hits. As a model bacteria, *Staphylococcus aureus* has been utilized, since it represents a leading cause of nosocomial infections, including prosthetic device-related infections and other severe pathologies (O'Gara 2007). In this contribution, the specific aim was to screen for anti-biofilm activity within an in-house collection of natural and naturally-derived compounds. As screening tool, an optimized assay based on redox fluorescence staining with resazurin was applied (Sandberg et al. 2009). All compounds were tested for their ability to prevent biofilms formation or to eliminate previously formed biofilms, the latest being the most relevant condition in the clinical context. Only one compound was identified as a hit, scoring above the activity threshold, in both exposure conditions. The compound was identified as a cinchonidine alkaloid derivative [11-triphenylsilyl-10,11-dihydrocinchonidine] (BIS-8) (Busygin et al. 2005) (**Figure 1**).

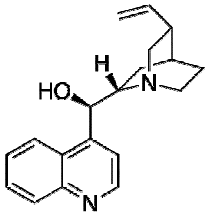

Figure 1. Structure of BIS-8 (11-triphenylsilyl-10,11-dihydrocinchonidine.

As expected, a more significant anti-biofilms effect was achieved by BIS-8 when preventing biofilms formation, rather than when killing the already formed ones. To characterize this potential lead compound, minimum inhibitory concentrations (MIC) values were measured. Effects not only on biofilms viability (using resazurin assay) but also on overall biofilm biomass (using crystal violet staining, as in Sandberg et al. 2008) were studied. Additionally, killing efficacy of BIS-8 was directly quantified by scrapping the biofilms off the wells and measuring their viable cell density in agar plate counts. With this data, Log Reduction parameter was estimated. Since MIC values were obtained in the micromolar range and a significant killing efficacy was achieved by BIS-8, this molecule seems to be a promising candidate as a lead anti-biofilm molecule.

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Keywords biofilms; *Staphylococcus aureus*; cinchonidine alkaloid

Analysis of the 2-Phenylethyl isothiocyanate present in *Brassica* leaves and their potential application as antimicrobial agent against bacteria strains isolated from Human and Pig gastrointestinal tracts

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Over the past 30 years, it has been shown that many classes of phytochemicals, particularly secondary plant metabolites including glucosinolates and more specifically their hydrolysis products (GHP), are important bioactives compounds in Human diet and potential therapeutic tools due to their antimicrobial activity. Recent epidemiological studies have reported several inhibition effects of GHP, against pathogenic bacteria's, specifically sulforaphane (a *Brasssica*-derived isothiocyanate from an important glucosinolate glucoraphanin largely present in cruciferous such as broccoli inflorescences) on *Helicobacter pylori,* the anaerobic bacteria responsible for human gastritis disease. Fewer studies have been conducted with other isothiocyanates. Thus, a study was set up to evaluate the effect of 2-Phenyethyl isothiocyanate (PEITC), a predominant isothiocyanate derived from glucosinolate named glucoranturtiin, particularly important in *Brassica rapa* (turnip-inflorescences, leaves, stems and roots) and *Nasturtion officinalis* (watercress), against pathogenic Gram-negative and Gram-positive bacteria, isolated from Human (clinical) and animal gastrointestinal segments. Using a disc diffusion method, different doses of PEITC were tested. The compound was dissolved in dimethyl sulfoxide (DMSO) and tested at 6 different concentrations using a standard disk-diffusion assay (0.015, 0.15, 0.75, 1.50, and 3.00 µmoles). Positive (commercial standards antibiotic) and negative controls (DMSO only) were always included in the tests. All the experiments were conducted in triplicate. The antibacterial activity was also assessed by the application of the percentage of relative inhibition zone (%RIZD) in relation to the antimicrobial effect of commercial standard antibiotics. The results showed that, there were clear concentration differences ($P < 0.05$) with respect to the *in vitro* growth inhibition effects as well as differences in the sensitivities of the individual bacteria. The antimicrobial activity of PEITC was always a dose-dependent. The results also showed that antimicrobial activity of PEITC was higher in Gram-positive, even higher than the antimicrobial activity of commercial standard antibiotic, and lower in Gram-negative. Nevertheless, our results are useful and suggest that PEITC might be useful in controlling Human and animal pathogenic bacteria's growth and therefore could be considered as alternative to, or in combination with, the current antibiotic-based controls used for treating pathogenic bacteria. However, further studies are needed to determine their MIC values, to evaluate their commercial and economical effectiveness, the eventual synergistic effects with others compounds, as found in the consumption of the Brassicaceae foods; toxicity bioassays must as well be performed.

Keywords: Phytochemycals, isothiocyanates, PEITC, antimicrobial, health.

Analysis of the antimicrobial activity and chemical composition of *Mentha pulegium*, *Thymus algeriensis* and *Juniperus phoenicea* essential oils from Morocco

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The great economic costs of deterioration and poisoning of food products by food pathogens, in addition to the increasing demand in recent years for safe and natural food, has provoked many researchers to explore new alternatives to traditional food preservation practices. Among these, Essential Oils (EOs) from aromatic and medicinal plants have received particular attention as potential natural agents for food preservation.

In this work we have evaluated the EOs of three aromatic plants (*Thymus algeriensis, Mentha pulegium* and *Juniperus phoenicea*) from Morocco. The EOs have been obtained by submitting the dried aerial parts of the plants to steam distillation for 2-3 h using a Clevenger-type apparatus. The composition of the EOs was analysed by GC-MS. Retention indices (KI) of all the constituents were determined by Kovats method by co-injection of the samples with a solution containing the homologous series of n-alkanes (C8–C20) on a DB-5 column. Identification of the components was made by comparing their retention indices and mass spectra with data published in the literature and by matching their recorded mass spectra with reference spectra in the computer library (NIST MS library Version 2.0). Some structures were further confirmed by available authentic standards. Quantification was computed as the percentage contribution of each compound to the total amount present.

The antimicrobial activity of the EOs has been evaluated against seven bacteria of significance importance. In so doing, the disk agar diffusion technique has been used against the selected microorganism: four Gram-positive, namely *Staphylococcus aureus* (CECT 239), *Enterococcus faecium* (CECT 410), *Listeria monocytogenes* serovar 4b (CECT 935) and *Listeria monocytogenes* EGD-e and three Gram-negative: *Salmonella* Enteritidis (CECT 4155), *Escherichia coli* serovar O157:H7 (CECT 4267) and *Pseudomonas aeruginosa* (CECT 110). Besides, we have determined the minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) by the tube dilution method.

Mentha pulegium EO showed a great similarity with essential oils coming from North Africa, being pulegone the major component accounting for about 70% of the oil. The EO of *Juniperus phoenicea* analyzed in this study had as main components α-pinene (24.90%), β-phellandrene (24.36%) and α-terpinyl acetate (12.85%). It is significant the high borneol content (23.48%) of *Thymus algeriensis* EO, which conjointly to linalool (8.99%), camphene (6.90%), carvacrol (7.76%) and β-caryophyllene (6.39%) were the major components, representing 53.52% of the oil. This chemical profile, rather different than other *T. algeriensis* EOs, even suggests the existence of a new chemotype.

The different EOs inhibited growth to variable extents, depending on the oil and the bacteria assayed. Attending to this, the major effectiveness was achieved by *Thymus algeriensis* followed by *Mentha pulegium* and *Juniperus phoenicea*. Gram-negative bacterial cells were, in general, more resistant to the presence of antimicrobials than Gram-positive ones. In addition, it was verified that *Pseudomonas aeruginosa* was the most resistant bacteria strain.

The results presented here contribute to the knowledge of the antimicrobial activities and chemical compositions of the tested EOs. Besides, our data support the possible use of the species of *Mentha pulegium*, *Thymus algeriensis*, and *Juniperus phoenicea,* in particular *Thymus algeriensis* EO, as potential natural agents for food preservation.

Acknowledgements: This study was supported by the CICYT (Project AGL 2009-11660) and Government of Aragón (Spain)-Fondo Social Europeo through Project DGA/Grupo de Investigación Consolidado (Project AO1). Thanks are also given to AECID, which provided to A. Ait-Ouazzou with a grant to carry out this investigation.

Keywords: *Thymus algeriensis, Mentha pulegium, Junipeurs phoenicea*, Essential oils, Chemical composition, Antimicrobial activity.

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Anti bacterial, Antifungal and Phytochemical analysis of Indian dietary spices

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The in vitro antibacterial and antifungal activities of a total of 48 extracts from 12 different dietary spices were investigated by various methods against three pathogenic bacteria *Bacillus subtilis , Staphylococcus aureus,* and *Escherichia coli* and two fungus *Aspergillus niger and Penicillium notandum. In-vitro* bacterial susceptibility test was conducted by agar plate-disc diffusion method. *In-vitro* fungal susceptibility test were done by Agar plate-well diffusion method, Fungal Radial Growth Assay and Fungal broth inoculation assay. Results have revealed that the extracts have broad spectrum activity against gram positive, gram negative bacteria and fungal strains. Hexane extracts of most of the spices have shown high activity in *in vitro* tests against the tested bacterial and fungal strains. Extracts of *Punica granatum*, *Myristica fragrans*, *Myristica fragrans* (nutmeg), *Trachyspermum copticum* and *Sesamunm idicum* demonstrated promising inhibitory action against *B. subtilis, E. coli and S. aureus* in different extracts. In case of antifungal assay conducted Methanolic and chloroform extracts of *Nigella sativa, Brassica nigra, Trachyspermum roxburghianum, Myristica fragrans* have shown a promising reduction in the radial mycilial growth in case of *Aspergillus niger.* Phytochemical evaluation revealed the presence of terpines, tannins, phenols, saponins and flavonoids. Of the 12 spices used in this study all showed considerable antimicrobial activity against one or more species of microorganisms tested. The antimicrobial activity is more significant in solvent extracts compared to aqueous extract in almost all the plants indicating that the active principle responsible for the activity is more soluble in organic solvents. The results of our present investigation look promising and clearly indicate the antibacterial and antifungal activity of the tested spices, though it still needs further investigation. The potential for developing antimicrobials from spices appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials

Keywords Spices, antimicrobial, phytochemicals

Anti-infective and free radical scavenging activities of phytochemicals present in some plant extracts

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The spread of drug resistant pathogens is one of the most serious threats to successful treatment of infectious diseases. Down the ages natural plant products and their extracts have evoked interest as source of alternative remedies for the treatment of many infectious diseases. Phytochemicals also acting as antioxidants have ability to control diseases caused by overproduction of radicals such as cancer, atherosclerosis, cardiovascular diseases and ageing. Present study reports the antibacterial efficacy and free radical scavenging activities of several plants such as *Bauhinia variegata*, *Tinospora cardifolia, Piper longum* and *Cinnamomum zeylanicum*. Various solvent extracts derived from test plants exhibited inhibitory efficacy against a group of pathogenic bacteria viz. *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* in disc diffusion assays. Zone of inhibition in *in vitro* assays ranged between 10-25 mm. Activity of extracts was compared with standard antibiotic discs (Vancomycin and Meropenem). Significant inhibitory efficacy was recorded in crude extracts derived from spice plants *P. longum* and *C. zeylanicum*. Petroleum ether, benzene, ethyl acetate and acetone extracts showed better bactericidal potential. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of effective extracts were found in the range of 0.43 – 7.1 mg/ml. Beta carotene linoleate model system was used for assessment of free radical scavenging activities. The percent antioxidant (AO) capacities of various extracts were recorded between 5-65%. The values were compared with the radical scavenging activity of standard antioxidant BHA (AO capacity 84%). Dose dependent antioxidant activity pattern was also observed in the assay. The % AO capacity gradually increased with increasing concentrations of the test extracts. Antioxidant activity was directly correlated with the amount of total phenolic contents in the extracts. In conclusion the present study demonstrated that phytochemicals present in test plants possess significant antibacterial and antioxidant activities.

Keywords: Antibacterial activity, free radical, antioxidant, MIC, MBC, *Bauhinia variegata, Tinospora cardifolia, Piper longum, Cinnamomum zeylanicum, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa.*

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Antibacterial activity of medicinal plant crude extracts against *Campylobacter* spp..

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Campylobacter, particularly *Campylobacter jejuni* contamination of poultry products, is an important pathogen causing gastroenteritis in healthy people, and with more severe complications in children, elderly, and individuals with underlying health problems. Because use of antibiotics in poultry production is prohibited, alternative pre-harvest interventions such as use of plant products and other natural products to reduce the carriage of *Campylobacter* in chickens are under extensive investigations. The study was conducted to determine whether ethanolic extracts of 60 medicinal plant species from 36 families which are effective plants on curing of diarreah human symptoms can in vitro inhibit the growth of 10 strains of *Campylobacter* spp. isolated from chickens and to evaluate whether the selected plant extract exhibiting the strongest anti-*Campylobacter* can prevent *Campylobacter* growth in vitro. In this study, an agar-well diffusion method was used to screen the antibacterial activity of the thai plants and to determine the minimal inhibitory concentration of the selected plant extracts. Of the 60 study plants, only 6 (or 10%) medicinal plants (*Terminalia chebula*, *Phyllanthus emblica*, *Senna alata*, *Mammea siamensis*, *Morinda citrifolia,* and *Piper betel*) inhibited all strains of *Campylobacter* examined. Ethanolic extracts of *Terminalia chebula* and *Phyllanthus emblica* showed the strongest activity against *Campylobacter* isolation (A2) with the MIC value as low as 25 mg/ml. Also *Terminalia chebula* demonstrated wash-out effect on Campylobaccter in the adjusted media broth. Therefore, ethanolic extracts of some medicinal plants have a high potential for further investigations to use as *Campylobacter* decontamination in poultry industry.

Keywords: Medicinal plants, Ethanolic extract, Antibacterial activity, Campylobacter, Chickens.

Table1 Inhibitory activity of medicinal plant extracts on 10 strians of Campylobacter growth by a well diffusion agar method

Campylobacter isolation[a]	Inhibition zones of medicinal plants on Campylobacter spp. in mean diameter (mm)[b]					
	Terminalia chebula	*Phyllanthus emblica*	*Cassia alata*	*Mammea siamensis*	*Piper betel*	*Morinda citrifolia*
A1	21±0.09	19±0.00	18±0.00	16±0.06	15±0.00	15±0.07
A2	22±0.00	17±0.05	15±0.00	15±0.06	15±0.00	15±0.07
A4	23±0.00	19±0.00	16±0.00	16±0.00	15±0.07	14±0.00
A6	21±0.00	16±0.00	16±0.00	15±0.00	17±0.00	15±0.00
B1	22±0.09	18±0.00	16±0.06	15±0.07	14±0.07	15±0.00
B2	22±0.08	18±0.05	18±0.01	15±0.00	15±0.08	15±0.00
B6	22±0.08	18±0.00	14±0.00	16±0.00	15±0.00	15±0.00
B7	24±0.04	18±0.00	15±0.00	16±0.00	17±0.06	15±0.00
B9	25±0.08	16±0.00	17±0.00	16±0.06	15±0.00	15±0.00
B10	22±0.04	19±0.05	16±0.00	15±0.06	15±0.07	15±0.00

[a]*Campylobacter* isolation from local backyard chickens
[b] Inhibition zone in mm.
Reported values on average are the diameter of inhibition zone in mm.

Antibacterial activity of an effective spice essential oil formulated in deodorant gels against skin bacteria involved in body odour

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Skin bacterial flora such as *Staphylococcus aureus, Staphylococcus epidermidis*, *Escherichia coli* including Methicillin-Resistance *Staphylococcus aureus* (MRSA) are able to metabolise axillary secretions that hence leading to body odour or axillary odour. Generally, a synthetic antibacterial agent used in various deodorant formulations is triclosan. Nowadays, bacterial resistance to triclosan has been developed. Essential oils distilled from aromatic plants are widely used as effective antimicrobial agents. In this study, spice essential oils distilled from *Citrus hystrix* DC. (kaffir lime) fruit peel, *Cymbopogon citratus* Stapf. (lemongrass) grass, *Cinnamomum zeylamicum* Nees (cinnamon) bark, *Ocimum basilicum* L. (sweet basil) leaves, *Alpinia galanga* (L.) Willd. (galanga) and *Zingiber officinale* Rosc (ginger) rhizome were determined their antibacterial activity against the mentioned skin bacteria using broth microdilution technique. The result revealed that cinnamon oil was the most active oil with possessing the lowest Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) against four bacterial strains. Therefore, chemical constituents of cinnamon oil were identified by Gas Chromatography / Mass Spectrometry (GC/MS). In addition, the lethal effect of cinnamon oil against *S. epidermidis* and *E. coli*, the representative body odour inducing Gram-positive bacteria and Gram-negative bacteria, respectively, was performed to determine a suitable concentration of cinnamon oil. The lethal curves showed that 9.375 µl/ml of cinnamon oil decreased 90 % of initial population of *S. epidermidis* and *E. coli* within 6 and 2 minutes, respectively. Therefore, 9.375 µl/ml of cinnamon oil was incorporated in three deodorant gel bases for axillary application. Physical stability of all formulations was studied by freeze-thaw cycling method. The most stable cinnamon oil deodorant gels was kept for 90 days at two storage conditions, room temperature with natural light and 45 °C without light. The lethal effect of deodorant gels exposed with *S. epidermidis* and *E. coli* for 1 hour was studied at day 0, 15, 30, 60 and 90. For all sampling days, cinnamon oil deodorant gels could decrease more than 90 % of initial bacterial population rapidly, within 1 hour. The significant difference of bacterial reduction ability between the gels kept at room temperature with natural light and the gel kept at 45 °C without light was not observed. In conclusion, cinnamon oil deodorant gels demonstrated a good ability to decrease the bacteria involved in body odour. The prominent antibacterial activity of cinnamon oil might be affected by eugenol and cinnamaldehyde, the major components in this essential oil revealed by GC/MS. The cinnamon oil deodorant gels possessed biological stability and hence contributing to be further developed as commercial product in the future.

Keywords spices; essential oils; cinnamon oil; body odour; deodorant gels; antibacterial activity; skin bacteria

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Antibacterial activity of aqueous extracts of three Algerian desert truffles against *Pseudomonas aeruginosa* and *Staphylococcus aureus* in vitro

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The aim of this study is to evaluate the in vitro effect of aqueous extracts of three Algerian truffles; *Terfezia claveryi, Terfezia leonis* and *Tirmania nivea,* on the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The antimicrobial activity of the three aqueous extracts was tested using agar well-diffusion and disk diffusion methods in addition to kinetic bacterial growth curves. The aqueous extracts of *T. claveryi* and *T. nivea* were found to possess a very powerful antibacterial activity against both *S. aureus* and *P. aeruginosa* using the well-diffusion, disk diffusion methods. Using 4 and 11% of the aqueous extracts of *T. claveryi* and *T. nivea* in the growth medium of *S. aureus* caused a significant inhibition of *S. aureus* growth by 86.48% and 99.09 respectively. The aqueous extracts of *T. claveryi* and *T. nivea* were found to cause a significant inhibition of the growth of *P. aeruginosa* by 71.11% and 100% respectively. Whereas, the aqueous extract of *T. leonis* did not show any antibacterial activity.

Therefore, *T. claveryi* and *T. nivea* can be considered as a source of natural antibiotics and can be used to isolate antibacterial substances against resistant bacteria such as *P. aeruginosa* and *S. aureus*.

Keywords: Algerian Truffles, Aqueous extract, antibacterial activity, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

Antibacterial activity of *Cytisus nigricans* L. extracts and their synergistic interaction with antibiotics

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Cytisus nigricans L., a member of family Fabaceae, is a perennial, herbaceous shrub-like plant widely distributed in Central and Southeast Europe. The biological activities of this plant are insufficient investigated.

In this work, the *in vitro* antibacterial activity of *Cytisus nigricans* L. ethanol, ethyl acetate and acetone extract and their synergistic interaction with antibiotics (gentamycin and cephalexin) were examined. Total phenol and flavonoid content presents in the extracts was determined, too. A broth microdilution method with resazurin was used to define minimum inhibitory concentration (MIC) against ATCC bacterial strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923, as well as, against clinical isolates of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Enterococcus faecalis*. Beneficial, synergistic herb-drug interactions, as an alternative approach to treat bacterial infection, were studied using checkerboard method. The combination effects were interpreted as fractional inhibitory concentration (FIC) index.

The activity of tested extracts varied depending on the species of bacteria and type and concentration of the extract. The comparative analyses showed that the most active was ethanol extract (MIC from 1.25 mg/ml to 20 mg/ml) followed by acetone extract (MIC from 2.5 mg/ml to >20 mg/ml) and ethyl acetate (MIC from 5 mg/ml to >20 mg/ml). The most sensitive bacteria were *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Bacillus subtilis*. The synergism was observed between ethanol and ethyl acetate extract with gentamycin and cephalexin. The acetone extract did not act synergisticly with tested antibiotics. FIC indices were in range from 0.36 to 0.44. The synergism was observed in relation to *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Enterococcus faecalis*.

Total phenol content was determined by Folin-Ciocalteu reagent and their amounts were in the range from 40.51 mg of GAE/g of extract to 86.31 mg of GAE/g of extract (expressed as gallic acid equivalent/g of extract). The amount of flavonoids, determined by aluminum chloride colorimetric method, was in the range from 34.74 mg of RU/g of extract to 167.96 mg of RU/g of extract (expressed as rutin equivalent/g of extract). The highest concentration of phenols were measured in ethanol extract and flavonoids in acetone extract.

The antibacterial property of *Cytisus nigricans* L. and synergism with antibiotics were tested for the first time. The activity could be attribute to presence of secondary metabolites from plant which have an impact on growth and metabolism of bacteria.

Keywords: antibacterial activity; Cytisus nigricans; synergism; total phenol content; total flavonoid content

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Antibacterial, antifungal and cytotoxic activities of two new flavonoids from *Retama raetam* flowers

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Antibacterial, antifungal and cytotoxic activities of two new flavonoids isolated from the flowers of *Retama raetam* were evaluated. Antibacterial and antifungal activities as evaluated using disc diffusion and microdilution broth methods. Cytotoxic activity was tested against Hep-2 cells using the MTT assay. The compounds 4',5, 7-trihydroxy-8-[3'', 3''-dimethylallyl]-flavone and 4', 5-dihydroxy-2'' , 2''-dimethylpyrano [5'', 6'': 5,6]- isoflavone were active against *P. aeruginosa and Escherichia. Coli* (7.81-15.62µg/ml) and they showed important antifungal activity, wile 4', 5-dihydroxy-2'' , 2''- dimethylpyrano [5'',6'': 5,6]- isoflavone displayed strong antifungal activity against *Candida* species (7.81 µg/ml). The tested compounds showed strong cytotoxic activity against Hep-2 cells. These two compounds can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens.

Keywords: *R. raetam* flowers; antibacterial; antifungal; cytotoxic, flavonoid, Compounds

Anticariogenic activity of active fraction from *Isertia* laevis against *S. mutans and S. sobrinus*

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Dental caries is considered a multifactorial, infectious, chronic, localized, posteruptive. and transmissible disease that leads to the destruction of dental hard tissue. The recognition of *Streptococcus mutans* as the major bacterial species involved in dental caries, has led to the implementation of prevention and control measures for the elimination or reduction of this microorganism in oral cavity. The fundamental goal of research on medicinal plants, is the search for substances or compounds with antimicrobial activity. The aim of this study was to evaluate the antimicrobial activity of fractions obtained of *Isertia laevis* by two methods against *S mutans* and *S. sobrinus*. The plant material was collected in Medina (Colombia) located at a height of 550 meters above sea level. From the ethanol extract of leaves of *I. laevis* fractions were obtained by two methods, extraction by vacuum column chromatography and extraction by continuous fractionation liquid / liquid. The evaluation of the antimicrobial activity of fractions against *S. mutans* and *S. sobrinus* was performed by well diffusion and bioautography assays. By vacuum column chromatography, only fractions of methanol and methanol-dichloromethane showed activity against *S. mutans* and *S. sobrinus*, with a minimum inhibitory concentration of 2 mg / well. In continuous fractionation liquid / liquid only the dichloromethane fraction showed activity against both microorganisms, with a minimum inhibitory concentration of 1 mg / well. Of the three active fractions were isolated C1 and C2 compounds, which showed a minimum inhibitory concentration of 0.4 mg / well for *S. mutans* and *S. sobrinus*, with halos of inhibition, respectively, 6.5 and 6.2 m.m. In conclusion, 1. The three active fractions of *I. laevis* showed activity against *S. mutans and S. sobrinus*, 2. C1 and C2 compounds present equally in the three active fractions showed activity on the two bacteria, 3. C1 and C2 compounds may correspond to structures of triterpene saponins and / or steroidal, and 4. The two extraction methodologies lead equally to obtain the active fractions.

Keywords: Dental caries, *S. mutans, S. sobrinus,* Antimicrobial activity, *Isertia laevis.*

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Antifungal activity of beetin, a ribosome-inactivating protein from *Beta vulgaris* L.

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Ribosome-inactivating proteins (RIPs) are a group of proteins with N-glycosidase activity present in a number of plants and some bacteria and fungi that remove adenines, in particular, the specific adenine from the sarcin/ricin loop of the large rRNA, in both prokaryotic and eukaryotic ribosomes, thus arresting protein synthesis at the translocation step. Apart from enzymatic activity and substrate specificity, very little is known about the biological function and role of RIPs. The potential function of RIPs as defense proteins has been supported by their enzymatic activity on ribosomal substrates isolated from several pathogenic microbes. Beetin is a type 1 RIP from sugar beet (*Beta vulgaris* L.) involved in plant systemic acquired resistance subjected to induction by phytopatogens. In the present study we demonstrate a direct inhibitory activity of beetin against the fungus *Penicillium digitatum*. Green mould is one of the predominant pathogens on citrus fruits worldwide. The results show that beetin promoted both, inhibition of mycelial growth and fungicidal activity toward conidia in *P. digitatum*. The use of different beetin concentrations and incubation times indicated that beetin interacted less strongly with conidia that with mycelial cells. In addition, microscopic observations show strong alterations of hyphal morphology produced by beetin. Once inside the target cell, beetin might exert its inhibitory function against the fungi by interrupting protein synthesis. These results support the hypothesis that beetin could have a role as anti-pathogen in the producing plant.

Keywords Ribosome inactivating protein (RIP); *Penicillium digitatum*; beetin; antifungal proteins

Antifungal activity of endemic *Origanum virens* from Portugal

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Origanum virens is widely used in Portuguese, and Mediterranem, cuizin. Althout it´s use as food condiment, it is also used in traditional medicin as antiseptic.

The antifungal activity of *Origanum virens* essential oil on *Candida albicans* ATCC 10231 and physico-chemical characterization of several extracts were evaluated. The essencial oil were obtained from the aerial parts of the plant by hydrodistillation and minimal inhibitory concentration (MIC) as well as the minimal lethal concentration (MLC) were used in order to assay the antifungal activity against *Candida albicans.* MIC and MLC values were 0,005% and 0,040% respectively, ranging from 0,005% to 0,080% of essential oil. Concentrations, lower than MIC values strongly prevent fungal growing (fig.1). The antifungal effect is time and concentration dependent, it´s observed after 6 hours of incubation for lower concentration (0,040%) and after 2 hours of incubation of essencial oil with *Candida albicans* for higher concentrations. It is difficult to attribute the activity of a complex mixture to particular constituents. Nevertheless, it is reasonable to speculate that the activity of this oil can be related to the presence of carvacrol and thymol.

The antioxidante activity of several extrats (methanol, ethanol and water) was also evaluated. Very promissing results were obtained, indicating a hight antioxidante activity of the extracts.

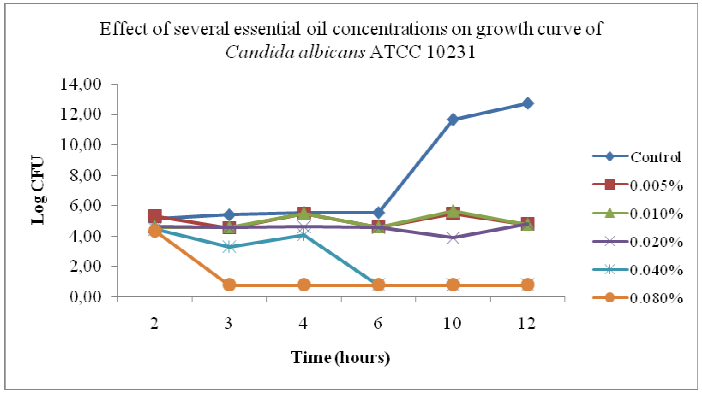

Figure 1: Effect of several essencial oil concentrations on growth curve of *Candida albicans*. The results were obtained by the macrodilution broth method, and minimal lethal concentrations (MLC´s) were performed acording to reference documents M27-A for yeasts (National Committee for Clinical Laboratory Standarts, 1997, 2002) after several hours of incubation of essencial oil with *Candida albicans*. Tests were carried out in triplicate.

Keywords *Origanum virens*; antifungal activity, physico-chemical characterization, antioxidant activity

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Antifungal activity of three essential oils against tomato (*Lycopersicon esculentum* Mill.) fungus strains *Fusarium oxysporum* f. sp. *radicis-lycopersici* in Côte d'Ivoire

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Pathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici* is a soil born fungus causing stem rot, wilt or roots rot on tomato. The use of the essential of *Ocimum gratissimum* (Combretaceae), *Zingiber officinalis* (Zingiberaceae) and *Pepper guinense* (Piperaceae) was investigated *in vitro* on the fungal radial growth and the germination of its spores. Three essential oils gave fongistatic activity against the fungus. The fungicidal activity against the pathogen was revealed to *O. gratissimum* and *P. guinense* at 0.2 µl/L. The best inhibition rate of the mycelium (>75%) and the germination (100%) of the spores was reached at 250 ppm with *O. gratissimum*. It is suggested that this variability might be the result of the nature and the volatile components of the oils. The possible contribution of the essential oils of *Ocimum gratissimum*, *Zingiber officinalis* and *Pepper guinense* to the reduction of tomato plant pathogenic fungus strain *Fusarium oxysporum* f. sp. *radicis-lycopersici* propagation is assessed.

Key words: Essential oils, *Fusarium oxysporum*, tomato, antifungal, Côte d'Ivoire.

Antimicrobial activity of *Alpinia purpurata* (Viell) Schum essential oil

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Plants have been a source of important chemical compounds that can be used for control of pathogens agents. Natural products are a source of active compounds and can be considered an alternative to synthetic molecules. The essential oils of several plant species have show antimicrobial and antifungal properties. The purpose of this study was to evaluate the antimicrobial effect of the inflorescence essential oil extracted from *Alpinia purpurata* (Zingiberaceae), a tropical ornamental plant widely cultivated in Pernambuco (Brazil). The essential oil was obtained by hydrodistillation in a Clevenger apparatus and analyzed by gas chromatography - mass spectrometry (GC-MS). The oil was tested against Gram-positive bacteria *Staphyloccocus aureus* (resistant and non-resistant strains) and *S. epidermis* and Gram-negative bacteria *Escherichia coli, Pseudomonas aeruginosa, Salmonella* sp., *Shigela* sp., *Proteus* sp. and *Klebsiella* sp. by minimum inhibitory concentration (MIC) test. The microrganisms were seeded in streaks on the surface of agar Müller Hington medium in Petri dishes incubated at 35 ^{0}C for 24 hours. The MIC determination method showed that the essential oil had a promising activity against all the bacteria tested. The lowest values of MIC were recorded for the Gram-positive species with values of 10µg/mL. For the Gram-negative bacteria MIC values of 1000 µg/mL were found. This is the first report of biological activity for this essential oil. Damaged inflorescence from plants are not suitable for commercial purposes, therefore, the production of this essential oil could be a way to make use of the discarded plants. The low MICs observed in this study, suggest that *A.purpurata* essential oil may be used in pharmaceutical preparations for treatment of infections caused by the bacteria tested.

Keywords: *Alpinia purpurata,* essential oil, antibacterial activity

Antimicrobial activity of Australian native plant essential oils against food-related bacteria

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Essential oils from 9 Australian native plants currently used by the flavour and cosmetic industry and traditionally used by indigenous Australians were evaluated for their antimicrobial activity against food spoilage and foodborne pathogens. The native plants studied were Eucalyptus Australiana *(Eucalyptus radiata ssp. radiata)*, Eucalyptus Blue Mallee *(Eucalyptus fruticetorum)*, Eucalyptus Blue Gum *(Eucalyptus globulus)*, Narrow Leaf Peppermint (*Eucalyptus radiata)*, Lemon Ironbark (*Eucalyptus staigeriana*), Lemon Scented Eucalyptus (*Eucalyptus citridora)*, Peppermint Eucalyptus *(Eucalyptus dives)*, Lemon Myrtle *(Backhousia citriodora)* and Anise Myrtle *(Backhousia anisata*). The major volatile compounds of these essential oils were analysed by Gas Chromatography Mass Spectrometry (table 1). Antimicrobial activity was determined using a microtitre plate assay and the Minimum Inhibitory Concentration (MIC) was determined. Twelve concentration levels of the essential oils were tested ranging from 0.0006% - 1.25%. The essential oils were evaluated against the following food spoilage microorganisms *Proteus vulgaris, Pseudomonas aeruginosa, Enterobacter aerogenes, Acinetobacter baumannii, Shewanella putrefaciens, Saccharomyces cerevisia, Geotrichum candidum* and food bourne pathogens *Bacillus cereus, Listeria monocytogenes, Escherichia coli* and *Staphyloccus aureus.* MIC values refer Table 2.

Table 1: Summary of major volatile compounds detected from essential oils of a range of Australian native plants

Plant essential oil	Major volatile compounds
Narrow leaf peppermint	1,8-cineole, alpha-terpineol, alpha-pinene
Peppermint Eucalyptus	Piperitone, alpha-phellandrene, p-cymene
Eucalyptus Blue Gum	1,8-cineole,alpha-pinene, lmonene, alpha-terpineol
Eucalyptus Blue Mallee	1,8-cineole,p-cymene,alpha-pinene
Lemon Scented Eucalyptus	Citronellal, citronellol, isopulegol
Lemon Ironbark	Limonene, terpinolene, beta-pinene
Eucalyptus Australiana	1,8-cineole, alpha-terpineol, limonene
Lemon Mrytle	Geranial (E-citral), neral (Z-citral)
Anise Mrytle	Methyl chavicol, anethole

Table 2: The concentrations(% v/v) of different EOs required to inhibit (MIC100) the growth of a range of food spoilage and pathogenic organisms

Plant Extract	*S.aureus*	*E.coli*	*B. cereus*	*P. vulgaris*	*P. aeruginosa*	*E. aerogenes*	*A. Baumannii*	*S. putrefaciens*	*L. cytogenes*	*G candidum*	*S. cerevisiae*
Narrow leaf peppermint	0.39	0.39	1.25	0.625	>1.25	1.25	0.313	0.313	0.625	0.156	0.078
Anise Mrytle	>1.25	>1.25	>1.25	>1.25	>1.25	>1.25	0.625	1.25	>1.25	0.156	0.078
Peppermint Eucalyptus	0.39	0.78	0.625	0.313	>1.25	0.625	0.313	0.313	1.25	0.156	0.156
Eucalyptus Blue Gum	0.39	0.78	1.25	1.25	>1.25	1.25	0.625	0.625	0.625	0.313	0.156
Eucalyptus Blue Mallee	0.78	0.78	1.25	1.25	>1.25	1.25	0.625	0.625	1.25	0.625	0.313
Lemon Scented Eucalyptus	0.098	1.56	0.313	>1.25	>1.25	>1.25	>1.25	1.25	0.625	0.625	0.313
Lemon Ironbark	0.78	1.56	0.313	0.625	>1.25	0.625	0.625	0.625	0.313	0.313	0.156
Eucalyptus Australiana	0.39	0.39	0.625	1.25	>1.25	1.25	0.625	0.625	0.625	0.313	0.313
Lemon Myrtle	0.156	0.313	0.156	0.313	>1.25	1.25	0.313	0.156	0.156	0.078	0.039

As revelaed lemon myrtle is the most effective essential oil against the tested microorganisms and is very effective against the yeasts requiring a concentration of less than 0.08%v/v for complete inactivation. Gram negative bacteria required a higher concentration for inhibition than gram positive bacteria. *Pseudomonas aeruginosa* required a concentration greater than 1.25%. Anise myrtle was the least effective against the tested microorganisms. Essential oils from Australian native plants have unique flavours which can be applied to different foods to create a range of innovative products. Results of this study indicate that essential oils such as lemon myrtle having a unique flavour and antimicrobial activity can be used to extend the storage life of fresh food. Consumers are increasingly looking for natural alternatives to chemical preservatives to meet this need natural antimicrobials such as essential oils have an important role to play in food preservation.

Keywords : Essential oils, Antimicrobial activity, Food spoilage, foodborne pathogens

Antimicrobial activity of Coriander Oil

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Plants contain numerous biologically active compounds, that can be seen as sources of agents to combat microbial diseases. For this reason, natural products are being screened for potential use against bacterial and fungal infections or contaminations. The antimicrobial properties of essential oils as well as compounds derived from them are exploited in such diverse commercial products as food preservatives, dental root canal sealers, antiseptics or feed supplements for lactating sows and weaned piglets. A few preservatives containing essential oils are already commercially available.

Coriander oil is obtained from seeds of *Coriandrum sativum* L. of the family Apiaceae. It is used in the food industry as a flavor ingredient agent and adjuvant. The oil is used as a flavor ingredient in the majority of the food categories, including alcoholic beverages, candy, pickles, meat sauce and seasonings. The oil is also used in consumer products such as soap, creams, lotion and perfumes.

In this work, the antimicrobial effect of coriander oil against reference strains was studied, which included three Gram-positive (*Bacillus cereus*, *Enterococcus faecalis* and *Staphylococcus aureus*), four Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*), three yeasts (two stains of *Candida albicans*, and one of *Candida tropicalis*) and against clinical isolates of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. The coriander oil was tested using the standard M7 and M27 microdilution method of the Clinical and Laboratory Standards Institute (CLSI), that were used to determine the minimum inhibitory concentration and minimum lethal concentration to the strains presenting susceptibility to this essential oil. The coriander oil presented antimicrobial activity in the tested concentration range against all tested bacteria and yeasts, except for *Pseudomonas aeruginosa*.

Considering the importance of *Staphylococcus aureus* pathogenesis and that this microorganism is the third most important cause of disease in the world among the reported foodborne illnesses, the development of strategies to control the survival and growth of *S. aureus* in food has been of great interest. So, additionally it was determined whether coriander oil is effective against *Staphylococcus aureus* in both planktonic cells and biofilms.

Keywords Antimicrobial activity; Coriander oil

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Antimicrobial activity of essential oil extracts of *Gongronema latifolium(Endel.) Decne* on bacterial isolates from blood streams of HIV infected patients in Lagos.

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Essential oils from *Gongronema latifolium* leaves *(Endl.) Decne* obtained by hydrodistillation were analyzed using Gas Chromatography/Mass Spectrophotometry (GC-MS).The oil was dominated by Fatty Acids Methyl Esters (FAMEs) which was characterized by high percentage of Phthalic acid (18.61%), stearic acid (4.63%), Palmitic acids (2.72%), Oleic acids (5.2%), arachidic acid (2.34%), and fumaric acid (2.22%). Monoterpenes including camphor, β – Cymene, and phytol as well as Sulfonamides, quinoline and carboxamide were also present.The essential oil as well as aqueous and ethanolic extracts of *Gongronema latifolium* leaves were evaluated for antimicrobial activity against bacteria isolated from blood streams of HIV patients in Lagos. Using agar diffusion method, the essential oil and the extracts showed moderate inhibitory activity against all the *Staphylococcus spp, Escherichia coli , Shigella spp , Salmonella spp, Klebsiella pneumonia ,Pseudomonas aeruginosa,Onchrobactrum anthropi and Candida albicans* . The zone of inhibition values recorded were comparable to control antibiotic ampicilline but less than that of Ciprofloxacin and Chloramphenicol .The MIC for essential oil ranged between 5µg/ml– 40µg/ml, while MBC also ranged between 5µg/ml – 40µg/ml,the MIC and MBC for ethanol extract ranged between 3.125mg/ml - 12.5mg/ml and 3.125mg/ml – 25.0mg/ml,while aqueous extract MIC range between 6.25mg/ml – 25.0mg/ml and MBC also ranged between 6.25mg/ml – 25.0mg/ml respectively.Extracts of *Gongronema latifolium* may be useful in ethnomedicine and in the treatment of blood stream infections in HIV patients.

Keywords: *Gongronema latifolium* ;essential oil;aqueous and ethanolic extracts;HIV related diseases;antimicrobial activity;fatty acids.

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Antimicrobial activity of four hydrocarbon monoterpenes acting alone or in combination with heat

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Plant essential oils (EOs) have attracted much interest because of their widespread use in perfumes, as the principal preservative in a variety of pharmaceutical or make-up products, sanitary products, and as food preservatives and additives. EOs are very complex natural mixtures that can contain about 20-80 constituents at significantly different concentrations. The monoterpenes are the most representative molecules, constituting 90% of EOs and comprising a great variety of structures. In general, oxygenated monoterpenes are significantly more active than hydrocarbons. Although chemical preservatives are mainly used to prevent microbial growth, recent studies are lauding their use as inactivating agents. Little is known about the mode of action and the capacity of these hydrophobic antimicrobial compounds to kill microorganisms as a function of the treatment conditions. Moreover, their mechanisms of microbial inhibition/inactivation are likely different. Concerning this subject, there are few studies that compare modes of action or antimicrobial spectrums under the same experimental conditions. On the other hand, since the doses to achieve microbial inactivation are expected to be very high when acting alone, their use in combination with other physical technologies such as heat or pulsed electric fields has been proposed. The most successful combined preservation treatments are those that achieve an excellent hurdle effect. Knowledge of the action mechanisms of each barrier would help establish the most effective treatment conditions.

The present study evaluates the antimicrobial activity of widespread EOs constituents, specifically 4 hydrocarbon monoterpenes (α-pinene, β-pinene, p-cymene and limonene), for control of growth and survival of spoiling and pathogenic microorganisms (*Enterococcus faecium, Listeria monocytogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella* Enteritidis *and Escherichia coli* O157:H7), their bactericidal activity as a function of the treatment medium pH, and possible synergistic effects in combination with mild heat.

Preliminary results obtained by using the filter paper disc agar diffusion technique would discount the use of hydrocarbon monoterpenes due to their poor inhibitory activity under the treatment conditions assayed. However, the evaluation of the bactericidal effect at pH 4.0 showed that all compounds assayed were highly effective against *E. coli* O157:H7 and *L. monocytogenes*. The use of selective recovery media has demonstrated that all hydrocarbon monoterpenes affected the cytoplasmic and the outer membrane causing sublethal injuries within the surviving population. Also the mild heat treatment assayed caused sublethal injuries in the cytoplasmic and the outer membrane of most surviving cells. Outstanding synergistic lethal effects were shown by combining heat (54 ºC/10 min) and the presence of hydrocarbon monoterpenes at very low concentration (0.2 µl/ml) as a function of the treatment medium pH and the microorganism investigated. Most combined treatments affected 3-4 extra Log_{10} cycles of cells, reaching the inactivation of more than 5 Log_{10} cycles of *E. coli* O157:H7 and *L. monocytogenes*. This work has also confirmed that cell envelope damage is an important event in microbial inactivation by heat and EOs constituents, helping us to establish successful combined preservation treatments.

Acknowledgements: This study was supported by the CICYT (Project AGL 2009-11660). Thanks are also given to AECID, which provided A. Ait-Ouazzou with a grant to carry out this investigation.

Keywords: essential oils, antimicrobials, foodborne pathogens, sublethal injury, heat, combined processes

Antimicrobial activity of hexane fraction of *Alchornea cordifolia* leaf.

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Alchornea cordifolia (Schum. & Thonn.) Muell. Arg. has been widely used in traditional medicine in West Africa for the treatment of several ailments most especially microbial infections. The search for antimicrobial agents must be continuous because of recurrent development of microbial resistance. Antimicrobial activity of the hexane fraction of the methanol extract of the leaf of *Alchornea cordifolia* was carried out using agar well diffusion and agar dilution methods against type organisms viz: *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 12600, *Escherichia coli* ATCC 11775 and *Candida albicans* ATCC 18804. The rate at which the hexane fraction was able to kill the organisms was also determined using standard method. Thin layer Chromatography (TLC) was used to separate the compounds of the hexane fraction. All the test organisms were susceptible to the fractions. The zones of inhibition showed by the fraction ranged from 11mm – 25mm. The Minimum Inhibitory Concentration (M.I.C) values were between 0.625mg/ml – 5.0mg/ml while the Minimum Bactericidal/Fungicidal Concentrations ranged from 1.25mg/ml – 10.0mg/ml. *Candida albicans* and *Pseudomonas aeruginosa* were totally killed after 2 hours while *Escherichia coli* was killed after 5 hours. *Alchornea cordifolia* leaf demonstrated important antimicrobial activity which may underlie their beneficial effect on microbial infection.

Keywords: Antimicrobial; *Alchornea cordifolia.*

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Antimicrobial Activity of Some Medicinal Plant seeds and fruit extract against gram positive pathogen from Fasialabad

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Synthetic drugs can produce health hazards and resistance in pathogens. This has led to discovery of safe and more effective natural sources like pharmacologically active peptides/proteins from medicinal plants. Antibacterial activity of *Hygrophila auriculata, Abrus precatoriu, Moringa oleifere, Croton tiglium, Withania somnifera, Psoralae corylifolia* and *Solanum nigrum* fruit extracts against *Bacillus megaterium* and *streptococcus equi,* was studied. Total proteins in the fruit and seeds of medicinal plants were extracted using extraction buffer and determined by biuret assay. Proteins/peptides were isolated using 80% ammiunm sulphate saturation. After desalting, protein/peptides were separated by gel filtration using sephadex G-200. Antibacterial activity against gram positive bacteria was determined by using disc agar diffusion method and was determined from zone of inhibition. These medicinal plants may be used for industrial scale extraction and isolation of antimicrobial compounds which may find place in medicine industry as constituents of antibiotics.

Key word: Antimicrobial Activity, Medicinal Plant from Fasialabad, disc agar diffusion method

Antimicrobial activity of *Thymus algeriensis, Rosmarinus officinalis and Eucalyptus globulus* essential oils acting alone or in combined processes

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An enormous research effort has been made lasts years in order to develop non-thermal methods of food preservation. Among them, Pulsed Electric Fields (PEF) has attracted much interest due to its capability of inactivating microorganisms without altering sensorial and nutritional properties of foods. However, the high resistance of some pathogenic microorganisms has restricted their use to preserve certain foods. Thus, nowadays, the search of new complex processes, combining traditional and emerging technologies, to reduce treatment intensity, increase food shelf-life and avoid loss of food quality is a challenge. In order to achieve an excellent hurdle combination it is necessary to know the mechanism of action of each barrier, leading to establish the best treatment conditions. The detection of sublethally injured cells is of great value when developing combined processes because it might increase hurdles lethality leading to synergistic effects. Heat and PEF have demonstrated to cause sublethal injuries in cell envelopes. When damages affect cell envelope hidrophobicity their combination with EOs might be a promising alternative.

The present study evaluates the antimicrobial activity against *Listeria monocytogenes* and *Escherichia coli* O157:H7 of 0.2 µl/ml of three EOs (*Thymus algeriensis*, *Eucalyptus globulus, Rosmarinus officinalis*) acting alone or in combination with a mild heat treatment (54 °C, 10 min) and a pulsed electric fields (PEF) treatment (30 kV/cm, 25 pulses), as a function of the treatment medium pH. The occurrence of sublethal injuries after heat, PEF and EOs treatments has also been evaluated.

Heat treatments were performed in a thermostatic bath under constant agitation. PEF treatments were carried out using equipment provided with a parallel-electrode treatment chamber where exponential-decay pulses were delivered. Survivors and sublethally injured cells were determined by counting the number of colony forming units after incubation of samples onto a non-selective medium and two selective media containing sodium chloride or bile salts.

Firstly, our results confirm that 0.2 µl/ml of the EOs tested were poorly effective against both microorganisms either suspended at pH 7.0 or 4.0. After 24 h of incubation at room temperature, only *Thymus algeriensis* reached an inactivation of approximately one Log10 cycle of E. coli at pH 7.0. Contrary to this, a synergistic lethal effect was observed when the three EOs were combined with a mild heat treatment. *Thymus algeriensis* was the most effective EO, allowing reaching the inactivation of 5 Log_{10} cycles of *E. coli* at both pHs, and approximately 3 Log_{10} cycles of *L. monocytogenes*. Regarding the combination of PEF and EOs, moderate synergistic effects were observed, reaching an extra inactivation of about 0-2 Log_{10} cycles of both microorganisms as a function of the medium pH.

The valuable synergistic effects observed between EOs and heat or PEF offer much potential to improve heat treatments, reducing treatment intensity, and thus adverse effects on food quality; or PEF treatments, achieving a higher microbial inactivation, and thus improving food safety.

Acknowledgements: This study was supported by the CICYT (Project AGL 2009-11660). Thanks are also given to AECID, which provided A. Ait-Ouazzou with a grant to carry out this investigation.

Keywords: essential oils, antimicrobials, foodborne pathogens, sublethal injury, heat, pulsed electric fields, combined processes

Antimicrobial and Anti Quorum Sensing activities of extracts from *Hypericum connatum*

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Guttiferae is a large family comprising more than 1000 species. The best-known genus is *Hypericum*, which encompasses various species used in traditional medicine around the world, as anticancer, antifungal, and antibiotic agents. *Hypericum connatum* Lam. is used in that traditional Argentine medicine, as a cardiac tonic and an astringent, as well as an antiviral agent, acting against lipid enveloped and non-enveloped DNA and RNA viruses, such as *Herpes simplex* virus.

We investigated the antimicrobial activity of an ethanolic extract of *H. connatum*, dried and re-dissolved in deionised water, against different Gram positive (*Bacillus cereus*, *Staphylococcus aureus*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Chronobacter sakazakii*) pathogenic microorganisms. The Anti Quorum Sensing (AQS) action was also evaluated. The *Chromobacterium violaceum* Quorum Sensing system was used for this assay. Quorum Sensing in this wild type strain of bacteria is directly linked to the production of the purple pigment violacein, due to the production and in response to autoinducer molecules, such as C6-acyl homoserine lactones and C4-acyl homoserine lactones. All strains, except *Chronobacter sakazakii,* were sensitive to the extract (from 50 µg to 100 100 µg). The most sensitive strain seemed to be the toxigenic strain *E. coli* DSM 8579, just when using 10 µg of extract. The extract (25 µg) exhibited Anti Quorum Sensing activity, causing the inhibition of the violacein production. Studies are in progress in order to isolate the possible active principle(s).

Antimicrobial and Anti Quorum Sensing activities of two typical *Brassica* cultivars present in the Campania region (Southern Italy)

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Brassicaceae are a family of vegetables widely diffused in the world, with every climate condition. The widest area of biodiversity is represented, in terms of species, by the Mediterranean countries. Many genera of *Brassicaeae* are used for human eating. The most important are *Brassica* (with many cultivars referred to cabbage, broccoli, rapeseed, etc) , *Sinapis* (mustard), *Raphanus* (radish), *Eruca* (rocket). In the Campania region (Southern Italy) two Brassica are present as typical products: rape broccoli "Friariello" (*Brassica rapa* var *rapa*) and "Torzella" (*Brassica oleracea* var *acephala*). Friariello exhibits an unique bitter taste and smell. Torzella (also called torza riccia or Greek cauliflower) is one of the most ancient brassica developed in the Mediterranean. Nowadays, it is cultivated mainly in the area of Acerrano-Nolano, in the province of Naples. We evaluated the antimicrobial activity of the ethanolic extracts of Friariello and Torzella leaves, against some Gram positive (*Bacillus cereus*, *Staphylococcus aureus*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Chronobacter sakazakii*) pathogenic microorganisms. The Anti Quorum Sensing (AQS) action was also assessed, using the *Chromobacterium violaceum* Quorum Sensing tester system and evaluating the inhibitory action of extract on the production of the microbial purple pigment violacein which, in this wild type strain of bacteria is linked to the production of autoinducer molecules, such as C6-acyl homoserine lactones and C4-acyl homoserine lactones, involved in the Quorum sensing activity. The extracts were assayed at amount ranging from 7.52 to 75.2 µg (friariello) and from 12.2 to 122 µg (torzella). Both extracts exhibited antimicrobial activity *versus* all strain tested (except that *P. aeruginosa*, resistant to the friariello extract), with inhibition halos ranging from 5 to 20 mm. It seemed of particular meaning the antimicrobial activity shown against the emergent pathogen *Chronobacterium sakazakii* . Both extracts exhibited Anti Quorum Sensing activity, causing the inhibition of the violacein production. Studies are in progress aimed to isolate the possible active principles and to identify their synergistic mechanisms of action.

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Antimicrobial and anti-adhesive properties of oregano (*Origanum vulgare*) against *Helicobacter pylori* strains

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*Helicobacter pylor*i is a bacterial pathogen that persistently inhabit the human stomach and thus colonization induces inflammation of the gastric mucosa that may progress into peptic ulcer diseases or adenocarcinoma. Bacterial attachment to stomach epithelium is considered the initial step for *H. pylori* pathogenesis. Moreover, *H. pylori* have the capacity to form biofilm where they have a remarkable protection against antimicrobial agents and the reduced susceptibility is recognized as an important factor in the persistence of chronic infections. Many therapeutic agents are used for eradication of this bacterium, but the widespread use of these agents has increased resistance among the isolated strains of *H. pylori*. In this sense, herbs and spices are used to increase the shelf lives of foods with a very well known antimicrobial effect. In fact many natural plant extracts have anti-*H. pylori* activity, including garlic, broccoli and cranberries. The aim of this study was to analyze the effect of oregano extract on i) growth of *H. pylori* and established *H. pylori* biofilms; ii) the gene expression of *H. pylori* biofilm cells. The reference strain NCTC11638 and clinical strain HP796, resistant to Clarithromicyn and Metronidazole, were used in this study. Oreganum vulgare (10 g) was boiled in 100 ml of distilled water to produce aqueous extracts. The antimicrobial activity of oregano extract was determined at 10 mg/ml by standard death-curve. One hundred µl of each bacterial suspension ($5x10^8$) were added to 900 µl of extract and incubated for 60 min in gas jars under microaerophilic conditions. To allow the formation of biofilm, both strains were grown in Petri dish with Mueller-Hinton Broth supplemented with 5% fetal calf serum extract and added a glass surface for adherence. The cultures were incubated under microaerophylic conditions for 48 h at 37ºC until formation of biofilm. Then the biofilm was transferred into a new plate with medium added of sub inhibitory concentrations of oregano extract (2.5 mg/ml) and was incubated for 26h. The effect of oregano extracts on *H. pylori* adhered to an abiotic surface was determined using plate counting. The morphologic changes were observed by optical microscopy in cells stained with crystal violet. The *lux*S gene, encoding for the autoinducer type 2, which is important for cell-to-cell signaling and *fla*A gene were analyzed at 90 min, 6h and 26h. For RNA extraction, the biofilm cells were treated with Trizol reagent. cDNA was performed with random hexamer and 200 U Moloney murine leukaemia virus reverse transcriptase.

The results showed that oregano extract have 100% of growth inhibition within 30 min. The sub inhibitory concentrations of oregano extract used in this study significantly inhibited the biofilm formation of reference and clinical strains with a decreased of 0.6 and 2 log units respectively. In established *H. pylori* biofilms the oregano extract decreased biofilm biomass ($p \leq 0.05$) of both reference and HP796 strain in more than 1 log units, additionally the extract was able to induce 100% *H. pylori* cocoid forms after 26 h of treatment. Respect to the effect on gene expression, the oregano extract showed an inhibition in the expression of *lux*S and *fla*A genes in *H. pylori* biofilm cells at 6 and 26h for both strains. The resistance of *H. pylori* to antibiotics has raised the demand for new agents with antimicrobial activity. This study identifies for the first time anti-*Helicobacte*r biofilm effects for *Origanum vulgare* and suggests that oregano extract can be envisaged as a food additive that could reinforce present therapies because it inhibits the growth the both planktonic and biofilm cell of *H. pylori* in addition to its inhibitory effect on the expression of genes responsible for biofilm formation *H. pylori*, considered a pathogenicity trait in chronic infections.

Keywords: Helicobacter pylori, origanum vulgare

Antimicrobial Anthraquinones Isolated from Lichen *Xanthoria parietina* Distributed in North of Iran

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Xanthoria parietina (L.)Th.Fr. (Teloschistaceae) has been considered as a potential source of anthraquinones biosynthesis with antiinfective properties.This study aimed to isolate and identify major anthroquinones biosynthesized by Iranian specimens of *X. parietina* and to assess their antimicrobial properties which support antiinfective property of this species growing in our geographical region. *X. parietina* specimens collected from the North of Iran were isolated using acetone-acetic acid solvent and analyzed by TLC and HPLC. They were identified using ^{1}H and ^{13}CNMR, FT-IR, MS, and UV absorbance. They were assayed against *Bacillus subtilis* (Gram-positive bacterium), *Escherichia coli* (Gram-negative bacterium) and *Pythium* sp. (Oomycetous fungus) by disc diffusion method. Fallacinal (5) (50%) and parietin (7) (33%) were the major anthraquinones in Iranian speciemns. They exhibited antimicrobial activity against *B. subt*ilis and *Pythium* sp. and had no antibacterial activity against Gram-negative bacterium.This study showed parietin and fallacinal were the major anthraquinones in Iranian specimens of *X. parietina* which have antimicrobial activity. Thus, it supported the value of *X. parietina* as a potential source of anthraquinones and antiinfective agents for potential drug development.

Keywords Anthraquinones; Antimicrobial

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Antimicrobial effect of a linen fabric made from genetically modified flax

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Flax (*Linum usitatissimum*) is an annual plant widely distributed in the Mediterranean and temperate climate zone. It has a long history of cultivation, and a great significance in medicine and industry. The three main products obtained from flax are the fibres produced from the stem, the linseed and oil extracted from the seeds. The properties of flax seeds and oil have been widely examined and have been proven to have many beneficial properties for human health. Recent modifications of flax enabled us to expand the use of flax fibers beyond the making of flax fabrics for clothing industry. Since it was suggested that reactive oxygen species are responsible for chronic wound pathogenesis the use of a modified flax fibers enriched in antioxidants for making of a wound dressing was proposed. It was shown, for a first time, that simultaneous use of fibres, oil emulsion and seedcake extract from genetically modified flax promotes healing of chronic skin ulcerations (Skorkowska-Telichowska 2010).

The inhibition of microorganism growth is important for wound protection against primary or secondary infection especially in case of chronic ulceration because quite often long - term wounds can become infected. Very often this is caused by a antibiotic- resistant bacteria and fungi and therefore new ways to combat a microbiological infection are needed. Here we present the data on influence of a two fabrics made from genetically engineered plants, proved to be effective as a wound dressing in a clinical trials, on a growth of a pathogenic bacteria and fungi *in vitro*. The first fabric (W) was made from flax plants enriched in antioxidants through overexpression of chalcone synthase, chalcone isomerase, and dihydroflavonol reductase genes (Lorenc-Kukula 2005). The second (M) was enriched with polyhydroxybutyrate which may promote proliferation of cells in high-density cultures by preventing apoptotic cell death (Wrobel-Kwiatkowska, 2009).

The antimicrobial activity of transgenic bandage was compared with that of the cotton and nontransgenic flax by determining their influence on microbial growth. Twelve clinical strains of bacteria (*Staphylococcus aureus* (n=4), *Staphylococcus epidermidis* (n=4), and *Enterococcus faecalis* (n=4)) and six clinical strains of pathological fungi (*Candida krusei* 153,*Candida krusei* 264, *Cryptococcus neoformans* 2110, *Trichosporon cutanem* 662, *Candida tropicalis* 252, *Candida albicans* 10231) were used for testing.

The transgenic fabric inhibited growth of all *S. aureus* and 3 of *S. epidermidis* isolates. All tested samples exhibited no inhibitory effect on *E. faecalis*. All samples inhibited bacterial growth only on contact surface with fibers. In case of pathogenic fungi the surface inhibition of growth of fungal colonies on flax fabric was observed for all samples. The M fabric was most effective against *Cryptococcus neoformans* and a W fabric against *Candida albicans*. Both transgenic fabrics inhibit the growth of pathogenic bacteria and fungi and are more effective than control flax and cotton fibres. The results of antimicrobial testing were compared with a biochemical testing of fibers in order to identify the fiber components responsible for antibacterial activity. Several chemicals with a potential antimicrobial activity were identified such as phenolic acids, terpenoids, sugar alcohols and fatty acids. At this stage we believe that antimicrobial effect of a flax fabric is a synergistic action of a many components found in flax fibers and a differences in activity toward particular strains derives from their sensitivity to particular components. To our knowledge is a first report on antimicrobial activity of a genetically modified flax fabric.

Keywords flax fibers, transgenic plants, *Candida*, *Cryptococcus, Staphylococcus aureus*

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Antimicrobial effect of carvacrol concentration on *Escherichia coli* K12 growth at different temperatures

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In response to consumer demands for fresher and natural food products, alternative technologies without relying neither on heat nor in chemical preservatives, are being developed for extending food shelf life. In this context, plant essential oils derived from herbs and spices are gaining interest for their potential as preservative ingredients given they have GRAS status and a notably antibacterial, antifungal and antioxidant properties that have been known since antiquity.

The aim of this study was to evaluate the antimicrobial activity of the oregano-derived volatile carvacrol on *Escherichia coli* K12 (CECT 433), grown in culture medium (Nutrient Broth), at two different incubation temperatures (30 and 37ºC). Specifically, it was studied the effect of concentrations between 0.04 and 0.14 µl ml^{-1} using the absorbance based microplate assay. The kinetic results were modelled with the Baranyi-Roberts and the modified Gompertz equations.

Regardless of the temperature, both the maximum specific growth rate (μ_{max}) and the lag phase (λ) showed to be dependent on the carvacrol concentration. An increase in the concentration produces a decrease in μ_{max} being 0.14 µl ml^{-1} a lethal concentration. Furthermore, a log-linear relationship between that parameter and the concentration of carvacrol can be found ($R \geq 0.900$). The lag phase is prolonged with increasing the antimicrobial concentration being only significantly longer for 0.12 µl ml^{-1} at 37ºC, and for 0.10 or 0.12 µl ml^{-1} at 30ºC. Comparing the results for both temperatures for each concentration, no significant differences were detected in the growth rate but the duration of the lag phase is always longer at 30ºC than at 37ºC ($P_{value} \leq 0.05$).

These results showed that carvacrol has a bacteriostatic effect on *E. coli* K12 growth so potentially it could be used to extend the shelf life and improve the safety margins of minimally processed foods, as primary or additional microbial control measure.

Keywords essential oils; carvacrol; antimicrobial activity; *Escherichia coli*; kinetic growth parameters

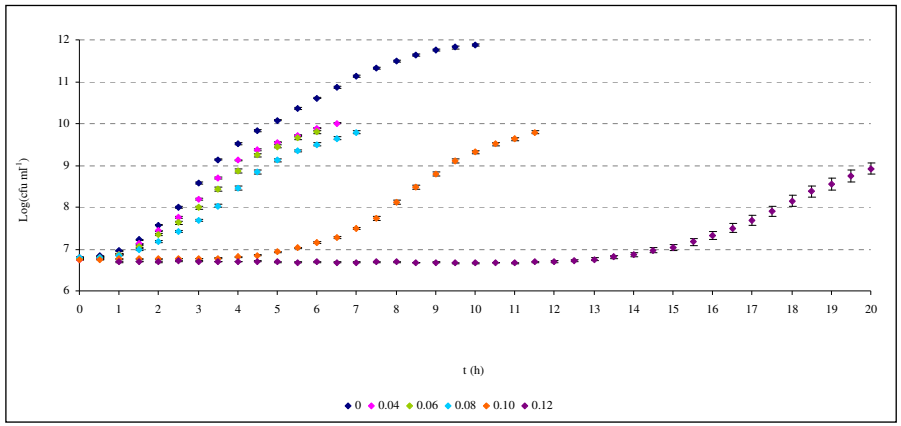

Fig 1. Growth curves of *Escherichia coli K12* in culture medium depending on the carvacrol concentration (µl ml^{-1}) at 30ºC. The coefficient variation was expressed by error bars.

Antimicrobial effect of the essential oils of some rare Nigerian medicinal plants

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The discoveries of plants with antimicrobial properties have proven effective for the control of bacterial and fungal infections. In Nigeria, there is a great flora and broad tradition in the medicinal plant for the control of these microorganisms. The antimicrobial activities of the essential oils of some these Nigerian medicinal plants, *Ageratum conyzoides* L, *Physalis angulata* L, *Argemone mexicana* (Papaveraceae), *Anchomanes difformis* (*Araceae*) and *Hygrophila auriculata* (Schum) were investigated against *Klebsiella pneumoniae, Bacillus subtilis*, *Staphylococcus aureus* (ATCC 24213), *Pseudomonas aeruginosa* (ATCC 9027) *Candida albicans* (ATCC 10231), *Candida stellatoidea*, *Candida torulopsis* and tested by using MIC and agar well diffusion. Positive and negative controls were included. Observed inhibition of these bacteria and fungi by essential oils of the whole part of the plants indicated that the antimicrobial properties are concentrated in both aerial and root parts of these plants. The MIC ranges between 2.0 – 4.0mg/ml, while the inhibition zones ranged from 8.0 ± 0.1 and 25.0 ± 0.3 which compete favorably with the positive controls. The conclusion was that the activity elicited by the essential oils of these plant on the selected bacterial and fungi makes them a strong and promising alternative for the development of probably new antimicrobial agents whose pharmacological and toxicological properties need to be assayed and quantified.

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Antimicrobial properties of the *Eragrostis viscosa* extracts

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Plants contain numerous biologically active compounds, many of which are known to have antimicrobial properties and can be seen as potential sources of agents to combat microbial diseases. In this work, the *in vitro* antimicrobial activity of different extracts obtained from *Eragrostis viscose* (grass harvested in Huíla province, Angola) was investigated. The disk diffusion method was used to evaluate antimicrobial activity, while the broth microdilution method was used to determine the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC). Their antimicrobial activities against reference strains, including three Gram-positive (*Bacillus cereus*, *Enterococcus faecalis* and *Staphylococcus aureus*), four Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*), three yeasts (two strains of *Candida albicans*, and one of *Candida tropicalis*) and against clinical isolates of methicillin-resistant *Staphylococcus aureus* were investigated. All the extracts inhibited more than one microorganism; moreover all of them presented antimicrobial activity against Gram-positive bacteria. These inhibitory effects can be considered relevant to the development of new agents for inclusion in the treatment or prevention of infections by the tested strains.

Keywords Antimicrobial activity; plant extracts

Acknowledgements

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Antimicrobial property of male accessory gland secretions in coconut beetle, *Oryctes rhinoceros*

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Bioactive molecules with antibacterial properties are being identified from various animal sources. Glandular secretions from many insect species exhibit antimicrobial action[1,2], are good sources of antimicrobial peptides. These substances provide the innate immunity mechanism in insects assisting their reproductive success[3]. Many antibacterial peptides are clinically important as antibiotic[4]. The present study report antimicrobial properties in the body secretions of the coconut beetle, *Oryctes rhinoceros*. Male accessory reproductive gland secretions (ARG) and haemolymph fluid (HF) from virgin male beetle were screened for antibacterial activity using disc diffusion sensitivity test. Five bacterial strains viz., *Bacillus subtilis, Klebsiella pneumoniae, Salmonella typhi, Proteus vulgaris and Pseudomonas aeruginosa* were tested against ARG and haemolymph fluid along with a reference antibiotic cyclofloxacin. 50 ul secretions and 20 ug cyclofloxacin were loaded individually on sterile discs placed on a bacteria seeded petriplates and incubated at 30 ^{O}C in BOD incubator. Antimicrobial action was identified as clear inhibitory zone developed around the loaded disc. Antimicrobial action of ARG and HF was identified against *B.subtilis, K. pneumonia* and *S. typhi.* However, *P. vulgaris* and *P. aeruginosa* were insensitive to both AG and HF of this insect. The experiment was repeated with virgin female and mated female insect reproductive secretions against *Bacillus subtilis*. Mated female spermatheca and accessory gland secretions exhibited antibacterial action in *B. subtilis* while the virgin female secretions failed to demonstrate antimicrobial activity. This suggests that the antimicrobial property of the male accessory gland is being transferred to female during mating. This support the view that male secretions provide antimicrobial protection simultaneously during reproductive process along with sperm contributions to female. The study also highlights the potential of insect sources to identify clinically important antimicrobial peptides.

Keywords: antibmicrobial; accessory reproductive gland; haemolymph; *Oryctes rhinoceros*.

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Antimicrobial Screening Effect of Rothmannia Plant

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Many medicinal plants have antimicrobial activity which is used to treat many health problems.

Rothmannia withifieldii has a good antimicrobial activity ranging from alkaloid, altonine, dimeric indole alkaloid are used as therapeutic for the treatment of parasitic protozoal infection, streptococcal infection like tonsilitis caused by *Streptococcus pyogenes.* Different part of the plant were extracted which include the stem extracts, root extracts and leave extracts.The phytochaemical test carried out revealed some major active constituents as flavonoids, tannins, saponins, steroidaaglycone, alkaloid were applied on the root cold water extract and ethanolic extracts of the stem. The ethanolic extracts of the root and stem showed greatest antibacterial activity against *Salmonella typhi* and *Sterptococcus pyogenes*.

Six bacterial isolates were tested on Rothmannia withifieldii plant to determine its antimicrobial potency. The bacteria used were *Psedomonas aeruginosa, Salmollela typhi, Escherichia coli, Proteus spp, Bacillus subtilis and Staphylococus aureus* and *Streptococcus pyogenes*.

The bacterial isolates tested showed a remarkable maximum zone of inhibition when tested on different extracts of the Rothmannia withifieldii plant. The Minimum bactericidal concentration (MBC) was determined and only *Salmonella typhi* show and *Streptococcus pyogenes.*

The minimum inhibitory concentration of the these extracts for bacterial specie tested fall within the range of 62.5 – 31.25 mg/ml. The plant of Rothmannia withifieldii when used as chewing sticks in some part of Africa have been found to cure upper and lower respiratory infections.

The results obtained in this research work shows that there were some justification in the use of the plant for treatment of typhoid fever, diarrhea and sore throat.

Key words: Antimicrobial activity, Rothmannia withifieldii, *Streptococcus pyogenes, Salmonella typhi*.

Antimicrobial substances from olive products: Implications on Health, Food, and Agriculture of glutaraldehyde-like compounds

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Olive oil, table olives, and different liquids generated during their processing contain notable quantities of various phenolic and oleosidic compounds with demonstrated antimicrobial activity. Among these substances, the dialdehydic form of decarboxymethyl elenolic acid either free (EDA), linked to tyrosol (TyEDA) or to hydroxytyrosol (HyEDA) display the strongest effect. The structures of these molecules include two aldehyde groups with the same skeleton that the antiseptic glutaraldehyde. This analogy should be the cause of our outstanding research in fields as different as human health, food processing, and agricultural pest control. In relation to human pathogens, we have demonstrated *in vitro* the bactericidal effect of olive oil against *Helicobacter pylori*, as well as the compound responsible for this activity, which is TyEDA at concentration as low as 1.3 μg/mL. Moreover, we have proven that added cells of food-borne pathogens (*Salmonella enterica* in mayonnaises or *Listeria monocytogenes* in salads) decay when olive oil is used instead of other vegetable oils, which imply that olive oil can be a hurdle component in foods like, salads, sauces, and others. With regard to food industry, we have disclosed the reasons why, in some instances, lactic acid bacteria fail to grow in table olive brines. The presence of HyEDA and EDA, among others compounds, provokes stuck fermentations and may give rise to spoiled products. Finally, in relation to plant pest management, there are different liquid byproducts -generated along olive oil and table olive production processes- that contain a noteworthy presence of molecules which are highly active against both bacterial (*Erwinia uredovora, E. amylovora, E. toletana, Pseudomonas savastanoi, P. syringae, Clavibacter michiganensis*), and fungal (*Alternaria* spp., *Pestalotiopsis dyospiri*, *Botrytis cinerea*, *Phytophthora cactorum* and *Colletotrichum acutatum*) phytopathogens. Mostly, the aqueous solutions used to preserve ripe olives before their darkening process show a notable activity without any deleterious effect against the vegetables assayed. All these research is being currently investigated *in vivo* with promising preliminary results.

Keywords olive; phenolic; antibacterial; antifungal; glutaraldehyde

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Antistaphylococcal activity of Violacein

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Violacein ([3-(1,2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1,3-dihydro-2H-indol-2-one]) is a purple pigment produced by free environmental bacterial species as *Pseudoalteromonas luteociolacea, Janthinobacterium lividum, Collimonas sp.,* and especially by *Chromobacterium violaceum.* This indole-derivative is synthesized from the condensation of two L-tryptophan molecules. Violacein synthesis is known to be induced under aerobic conditions and in response to *quorum sensing*, although physiological function of violacein is not yet clarified. This compound has shown to have several biological properties including antitumoral and apoptosis inducing functions in cancer cells, antioxidant, leishmanicidal, antimalarial, trypanocidal, antifungal, antibacterial and antiviral activities. As antibiotic, violacein has significant activity against *Mycobacterium tuberculosis.* In this context, the aim of this work is to investigate the antibiotic activity of violacein against *Staphylococcus sp* medical bacterial strains, their growth inhibition kinetics as well as the action mechanism. For this, the strains were further assayed regarding the minimal inhibitory concentrations (MIC), the minimum bactericidal concentration (MBC), time-response growth curves *in vitro* in the presence of violacein, transmission electron microscopy and determining changes in protein expression using 2D gels. This pigment exhibited a higher inhibitory effect against *Staphylococcus aureus* and *Staphylococcus epidermidis*, including methicilin-resistant *S. aureus* (MRSA) and vancomycin intermediary-resistant *S. aureus* (VISA) strains, which are a concerning in terms of hospital-acquired infections. The electron micrographs show changes in the cell wall in *S. aureus*, indicating this structure as a possible action site of violacein. Preliminar proteomic analysis identificated differentially expressed proteins by *S. aureus* in the violacein presence or absence, that indicated disturbances in bacterial metabolism, inhibiting some pathogenicity factors, additionally, more substantial investigations are needed to confirm this data. This results suggest that violacein is an antimicrobial with bactericidal activity and potential use in *Staphylococcus aureus* control, an important human pathogen, among other microorganisms.

Keywords: violacein, antimicrobial activity, *Staphylococcus aureus*

Antmicrobial flavonoids isolated from mango leaves

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Five flavonoids namely (-)-epicatechin-3-*O*-β-glucopyranoside(1),5-hydroxy-3-(4- hydroxylphenyl) pyrano⌈3,2-g⌋chromene-4(8H)- one. (2), 6-*p*-hydroxybenzyltaxifolin-7-*O*-β-D glucoside (Tricuspid) (3), Quercetin-3-*O*-α-glucopyranosyl-(1→2)-β-glucopyranoside (4) and (-) epicatechin (2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H chromene-3,5,7-triol) (5) were isolated from leaves of mango (*Mangifera indica* L.). Antimicobial activity of these compounds was evaluated against five fungal species [*Alternaria alternata, Aspergillus fumigatus, Aspergillus niger Macrophomina phaseolina* and *Penicillium citrii*] and four bacterial strains [*Lactobacillus* sp., *Escherichia coli*, *Azospirillium lipoferum* and *Bacillus* sp.]. Six concentrations viz. 100, 300, 500, 700, 900 and 1000 ppm of each of the five flavonoids were employed by means of poisoned medium technique. All the concentrations of the five test flavonoids significantly suppressed the fungal as well as bacterial growth. In general, antifungal activity of the flavonoids was gradually increased by increasing their concentrations. The highest concentration of 1000 ppm of compounds 1–5 reduced the growth of different target fungal species by 63–97, 56–96, 76–99, 76–98 and 82–96%, respectively. Compound 1 exhibited least antibacterial activity resulting 7–75% reduction in growth of different bacterial species. Compound 5 showed the highest antibacterial activity and its different concentrations reduced the bacterial growth by 45–99.9%. *A.*

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Application of hand spray using peppermint oil against *Aspergillus niger* on rubberwood

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Antifungal activity of hand spray using peppermint oil against *Aspergillus niger* indentified from rubberwood was investigated. The broth dilution method was employed to determine the minimal inhibitory concentration (MIC) by mixing peppermint oil at 100-400 μg ml^{-1} . It was found that peppermint oil was the strongest inhibitors with the MIC of 300 μg ml^{-1}. Mold test of *A. niger* on treated rubberwood (spray with peppermint oil at 300 μg ml^{-1} was then conducted according to the ASTM D4445-91. After 12 weeks of exposure at 25 °C and 100%RH, the percentage of mold (base on control) was determined. The results indicated that rubberwood treated with hand spray using peppermint oil was capable of providing a complete protection from *A. niger* growth on rubberwood. These finding suggested that hand spray using peppermint oil has good potential for protecting rubberwood products from the attack of mold (*A. niger*).

Keywords peppermint oil; *Aspergillus niger*; rubberwood

Assessment of Antibacterial Potential of Noxious Aquatic Algal Weed *Pithophora oedogonia* (Mont.) Wittrock

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In India, traditional medicine plays an important role in the primary health care. Most of the botanical remedies used as medicines are obtained from the terrestrial habitats, but the lower aquatic flora are also rich source of structurally novel and biologically active metabolites (secondary/primary) that creates great attention to the pharmaceutical industry as a source of antimicrobial drugs. To date, several unique compounds of aquatic origin bestowed with various biological activities have been extracted and characterized some others are under investigation. To cope up the increasing demand for therapeutic drugs from the natural products, greater interest has now arisen in algae. The first investigation on the antibiotic activity of an alga was carried out by Pratt *et al.* (1944). Amongst several such algae, *Pithophora oedogonia,* a green filamentous nuisance aquatic algal weed, which forms a cottony matt in the water bodies, is also now known for its bioactivity. The methanolic extract of *P. oedogonia* after defatting with petroleum ether and chloroform at 40-60^{o}C has been found to exhibit strong activity against two gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and three gram negative bacteria *Escherichia coli, Helicobacter pylori* and *Salmonella typhae*. The antibacterial activity was evaluated using the agar diffusion technique in petridishes. The indicator bacteria were inoculated on Mular- Hinton agar plate supplemented with 25 µl of extract at fixed location. After incubation for 24 hours at 30^{o} C a clear inhibition zone on disc measuring around 18.1±0.44 mm in diameter evidenced the antibacterial activity. This inhibitory effect was less common in gram negative than gram positive bacteria. Phycochemical screening of the extract of filamentous green alga *Pithophora* indicated the presence of secondary metabolites, such as phenolic compounds, terpenoids, tannins and alkaloids in the eluted fraction of methanolic extract, which resulted into excellent zone of inhibition in contrast to those of standard antibiotics. The results of this study reveal fruitful utilization of *P. oedogonia* causing great nuisance in the municipal water supply and recreational reservoirs.

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Bactericidal activity of essential oil of *Cymbopogon citratus* (DC) Stapf. *Poaceae-Gramineae* from Angola

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Cymbopogon citratus (DC) Stapf.*Poaceae – Gramineae*, is a native herb from India and is also cultivated in other tropical and subtropical countries, namely Angola. It is consumed as an aromatic drink and used in traditional cuisine for its lemon flavour, but it is also employed in popular medicine. Infusions or decoctions of dry leaves have been used as stomachic, antispasmodic, carminative and antihypertensive agents. In many countries it is used to treat feverish conditions and as a relaxant and sleeping aid. It helps with emotional states and it is an antidepressant agent. Studies on extracts from *C. citratus* leaves have demonstrated anti-inflammatory, hypotensive, vasorelaxating and diuretic activities, efficiency against oxidative damage and also cancer chemopreventive properties. *C. citratus* leaves essential oil is often applied for the flavour and fragrance industries and in pharmaceutical industry is used as a source of phytochemicals for the development of new drugs. The need to find effective new drugs in combating microrganisms has stimulated a microbial search for alternative sources of compounds with antimicrobial activity. The plants by submitting a much higher molecular diversity that is derived from synthetic products are an excellent source of seeking new antimicrobial drugs. The antimicrobial properties of essential oils arouse interest constitute an alternative to the requirement of consumers on the use of natural food additives. Antimicrobial activity evaluation of essential oils is difficult because of its volatility, insolubility in water and complexity. Essential oils are hydrophobic and with high viscosity. These properties may reduce the ability of dilution or cause an unequal distribution of oil through means even using a suitable solvent. This work was to assess the activity of essential oil *Cymbopogon citratus* (DC) Stapf. *Poaceae-Gramineae* from Angola front strains ATCC and strains with demonstrated anti-microbial resistance to various drugs. Antibacterial activity assessment was made by broadcast agar method, according to the methodology proposed by CLSI/NCCLS. The tests were carried out in triplicate, accompanied by a positive control with antibiotics such as metilcilin (5µg); penicillin (10µg); augmentim (30µg) to the strains Gram-positive *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, and ceftazidime (30µg); ciprofloxacina (5 µg), nitrofurantoína (300µg), gentamicin (10µg) in Gram-negative strains *Eschericihia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 25933; and a negative control with DMSO. Also evaluated the minimum concentration inhibitory (MIC) an injunction of essential oil given the strains showed sensitivity to these components such as *Staphylococcus aureus, Staphylococcus epidermidis* and *Escherichia coli*. With the same agar method, also evaluated the effect *in vitro* of the essential oil in nosocomial multirresistent strains that demonstrated a very good results in *S. aureus* and *St. epidermidis*. The results suggest that this essential oil and its majority component have the potential to use against bacterial infections, particularly skin.

Keywords *Cymbopogon citratus*, bactericidal activity, essential oil, agar method, minimum concentration inhibitory, *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella penumoniae, Proteus mirabilis*

Bacteriostatic effect of cocoa powder rich in polyphenols to control *Cronobacter sakazakii* proliferation on infant milk formula.

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International attention is given to the safety of food for infants and young children and, at the same time, achieving attractive nutritional and flavouring value of these infant products (WHO, 2008). Various technologies associated with the addition of complementary ingredients to generate innovation of an assortment of flavors, textures, and functional attributes are applying in milk derivatives. In this sense, several studies reveal results on the preservative action of these natural ingredients such as e.g. cinnamon, anise, cocoa, or vanillin (Cooper et al., 2007).

In the present research work, we propose the study of growth behaviour of *Cronobacter sakazakii* on milk formula (MF) supplemented with different cocoa powder concentration. The main objective of the present study was to determine the possible inhibitory/inactivation effect of cocoa powder on the *Cronobacter sakazakii* proliferation at 25ºC in a milk formula (MF).

The study was carried out on MF supplemented/not supplemented at three different cocoa powder rich in polyphenols (CCX) concentration: 1, 2.5 and 5% (w/v). Experimental data were fitted to Gompertz equation to analyze the growth behavior of *Cronobacter sakazakii* at 25ºC in MF and in MF supplemented with CCX.

The lower the specific growth rate (μ_{max}), the higher the inhibitory effect of the CCX on *C. sakazakii* growth. However, there were not significantly differences between not supplemented/supplemented MF μ_{max} values at different CCX concentration. In the same way, not significant differences were showed between *C. sakazakii* final load (Log N*f*) in different beverages after incubation period. On the other hand, the higher the lag phase (λ), the higher the bacteriostatic effect of the substance. In the present study, it was observed, that the CCX concentration contributed significantly to extend lag phase duration at 5% (w/v) supplementation. In MF-CCX 5%, λ (h) showed significantly higher value (7.789±0.122) than the other not supplemented/supplemented (1% and 2.5 % CCX) milk formulas (3.230±0.512). Other authors have reported the effect of cocoa powder as an ingredient revealing bacteriostatic effect on different microorganisms growth. Gabis and Langlois (1967) reported that the growth of several test organisms in milk was retarded by the presence of cocoa powder. Pina et al. (2009) obtained an inhibitory and bacteriostatic effect to face *Bacillus cereus* growth at 5, 20, and 37 ºC by addition of cocoa powder (2.5% (w/v)) in skim milk substrate.

Consequently from the present study, there is no inhibitory effect of CCX on *C. sakazakii* growth in different MFs, but a significant bacteriostatic effect was observed with 5% supplemented CCX milk formula according to the growth parameters values obtained. These results could be valuable avoid microorganism growth, for instance in pasteurized products that should be kept under refrigeration in the event of a cool chain breakage..

Keywords cocoa powder, growth inhibition, antimicrobial effect, *Cronobacter sakazakii*

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Biochemical Comparison of Two Ecologically Distinctive Specimens of *Xanthoria parietina* in North of Iran for their Antimicrobial Pigments

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Biochemical comparison of two ecologically distinctive specimens of lichen *Xanthoria parietina* (L.) Th. Fr. (Lecanorales:Teloschistaceae) in north of Iran was investigated to find out how bioactive pigments might be changed under different climatic conditions. Acetone extracts of specimens were subjected to biochemical fractionation and pigment content was evaluated for its quality and quantity. Specimens with high amounts of bioactive pigments, mainly anthraquinones, were fractionated into three parts, F1-F3, and then tested for their antimicrobial activity against some Gram-negative and Gram-positive bacteria and fungi by using agar diffusion method. Statistical analysis indicated that only F3 did show relatively reliable antimicrobial activity against Gram-positive bacterium *Bacillus subtilis* and Oomycetous fungus *Pythium* sp. In contrast, none of fractions did show antibacterial activity against Gram-negative bacteria and some filamentous fungi. At the final step, F3 (benzene-dissolving fraction), previously supposed to have anthraquinones, was chosen to be more fractionated and tested for bioactive pigments. Obtained from F3, subfractions 5 and 7 were identified as anthraquinones by UV absorbance pattern and thin layer chromatography (TLC) characteristics. High performance liquid chromatography (HPLC) chromatogram indicated them as main peaks with share of 50% and 33% of total peak area, respectively. Subfractions 5 and 7 showed antibacterial activity against Gram-positive bacterium *B. subtilis* and antifungal activity against *Pythium* sp. This study showed that specimens distributed in highlands exposed to sun with non-saturated atmosphere have considerable amounts of anthraquinones which can be considered as potential antimicrobial agents for drug development.

Keywords Antibacterial; *Xanthoria parietina*

Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combination with heat

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Many natural plant components and extracts have been used in food preservation for thousands of years. In particular, essential oils (EOs) and their constituents have been widely investigated for their antimicrobial properties. Among the great variety of EOs and their components, those contained in citrus fruits seem to arouse special interest because they are considered GRAS (Generally Recognized As Safe) and their addition does not usually involve unpleasant changes in the sensorial properties of food products.

Although the efficacy of EOs from citrus fruits and their individual components has been proved against molds and yeasts, little has been studied on their effect on the inhibition and inactivation of foodborne pathogenic bacteria. Moreover, antimicrobial activity of EOs depends on their chemical composition, but to the best of our knowledge, little is known about which constituents or mixtures of them are mainly responsible for the antimicrobial properties of citrus fruit EOs. Therefore, this study investigated the chemical composition of commercial orange, lemon and mandarin EOs, their effectiveness *in vitro* on survival and growth of three Gram-negative (*Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Pseudomonas aeruginosa*) and three Gram-positive (*Staphylococcus aureus*, *Listeria monocytogenes*, and *Enterococcus faecium*) spoiling and pathogenic bacteria, as well as possible synergistic lethal effects in combination with mild heat.

The composition of the EOs was analysed by GC-MS. Retention indices (KI) of all the constituents were determined by Kovats method. Identification of the components was made by comparing their retention indices and mass spectra with data published in the literature and by matching their recorded mass spectra with reference spectra in the computer library (NIST MS library Version 2.0). Some structures were further confirmed by available authentic standards. The antimicrobial activity of the EOs was evaluated by the disk agar diffusion technique and the tube dilution method, which allowed us to determine the minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC).

Regarding chemical composition, GC-MS analysis allowed for the identification of 65 compounds as main constituents, being limonene the most frequently present in the three EOs (59-85%). Results obtained by using the disc agar diffusion technique pointed out that mandarin EO was the only one with growth inhibitory activity. Since a significantly higher proportion of oxygenated monoterpenes in mandarin EO was found, these compounds might be involved in its strongest antimicrobial activity.

The determination of the MIC and MBC confirmed the broadest spectrum of action to be that of the mandarin EO. Nevertheless, although orange and lemon EOs were practically unable to inhibit or inactivate Gram-negative bacteria at the tested concentrations, they showed significant antimicrobial activity against Gram-positive bacterial cells. Therefore, despite the similar plant origin of the citrus fruit EOs assayed, our results demonstrated great differences within the chemical composition of the three EOs and their antimicrobial activity.

On the other hand, a preservation process based on the use of citrus fruit EOs with mild heat might be used to obtain safe products whose nutritional and sensory properties would be less affected than after traditional heat treatments. In this sense, the simultaneous application of 54°C for 10 min in the presence of 0.2 µL/mL of each one of the three EOs in citrate-phosphate buffer was assayed. The results showed that this combined process caused a greater inactivation than the sum of that obtained by both methods acting separately: even though the heat treatment inactivated less than 1 and 2 $\log_{10}$ cycles of *Escherichia coli* O157:H7 and *Listeria monocytogenes* EGD-e respectively, more than 5 $\log_{10}$ cycles of inactivation were achieved after the combined process. Consequently, the potential of these citrus EOs in the design of synergistic combined treatments was demonstrated. Due to their flavor and liquid consistency, the application of these citrus EOs might be specially recommended to improve the pasteurization process of fruit juices.

Acknowledgements: This study was supported by the CICYT (Project AGL 2009-11660). Thanks are also given to Gobierno de Aragón, which provided L. Espina with a grant to carry out this investigation.

Keywords: citrus essential oils; antimicrobial activity; foodborne pathogens; sublethal injury, heat, combined process

Competitive interactions as a new source of fungal metabolites.

Olivier Schumpp, Nadine Bohni, Samuel Bertrand, Jean-Luc Wolfender, Stéphanie Schürch and Katia Gindro

Antimicrobial activity of fungal derived compounds has been demonstrated more than hundred years ago and some of today's most recent medical drugs used as antimicrobial compounds still derive from fungi. Indeed, fungi are well known to constitutively produce a very large diversity of secondary compounds and remain therefore a promising source for research of new drugs derived from natural products (NPs).

In spite of their plethoric availability however, research of new lead components from NPs is poorly efficient: resolution of NPs' elaborated structures is time consuming and complexity of natural extracts makes bioguided isolation of pure lead difficult. As a result, pharmaceutical companies massively favour synthetic libraries as a source of new compounds.

In order to optimise isolation of NPs with relevant biological activity, we induced the production of new metabolites using biological stresses as a stimulus. Fungi from different taxonomical groups were challenged with other microorganisms on petri dishes. Four interaction-behaviours referred as interaction types were observed and hundreds of fungal combinations were classified accordingly. In each case, pure cultures of both fungi and excised interaction zone of co-culture were extracted separately and metabolic profiles were compared by Ultra High Pressure Liquid Chromatography coupled with Electrospray Ionisation Time of Flight Mass Spectrometry (UHPLC-ESI-TOFMS). A surprisingly large diversity of new molecules specifically induced upon interaction was observed with some fungal strains while other fungal interactions did not induce the production of any detectable new low molecular metabolites. Because they are synthesised in response to microbial interactions, these compounds are likely to possess defensive and/or antimicrobial properties. By analogy with the well-known phytoalexins synthesised in plants as plant defence responses to microbial attack, we termed these substances mycoalexins (Glauser et al. 2009). UHPLC-ESI-TOFMS is a highly sensitive analytical method for extract profiling that provides the exact mass of any compounds for dereplication purposes. The separation can be geometrically upscaled for semi-prep LC-MS fractionation and isolation of mycoalexins (Bohni et al., unpublished results). Thus, comparison of metabolic profiles between challenged and unchallenged fungal extracts accelerates dramatically the isolation procedure and only semi-pure mycoalexins, putatively active as antimicrobial compounds, enter the biological test phase.

In combination with identification using highly sensitive microflow NMR (CapNMR™) equipped with automated sample injection (One Minute-NMR™), the whole process turns fungal induction into a competitive source of secondary metabolites for future therapeutic drugs.

Glauser, G., Gindro, K., Fringeli, J., de Joffrey, J.-P., Rudaz, S., Wolfender, J.-L. (2009) Differential analysis of mycoalexins in confrontation zones of grapevine fungal pathogens by ultra-high pressure liquid chromatography/time-of-flight mass spectrometry and capillary nuclear magnetic resonance. J. Agric. Food Chem. 57: 1127-1134.

Keywords: Mycoalexin, Mycelial interaction, Ecological strategy, Drug discovery, Natural product.

Curcumin: a natural antibiofilm agent

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Biofilms are becoming increasingly problematic in both medical and industrial settings, prompting an urgent need to discover new agents that inhibit and/or eradicate these microbial communities. Screening natural compounds for antibiofilm activity is proving a promising approach. In this study we characterized the antibiofilm activity of curcumin. Curcumin is an extract of turmeric known to exhibit antimicrobial properties, however, its antibiofilm activity has yet to be thoroughly investigated. We find that curcumin reduces biofilm formation by *Staphylococcus aureus* and *Escherichia coli* and in addition, can effect removal and killing of mature biofilms. Preliminary data indicates that curcumin imposes membrane damage in *S. aureus* and morphological changes in *E. coli* cell wall which may possibly mediate the mechanism by which curcumin exerts its anti-biofilm activity. Our findings evidence the potential of curcumin to serve as a natural antibiofilm pharmacological agent.

Keywords: biofilm; curcumin; antimicrobial

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Effect of Flavanol-Rich Cocoa Powder on the Growth of *Bacillus cereus* Spores in Reference Media

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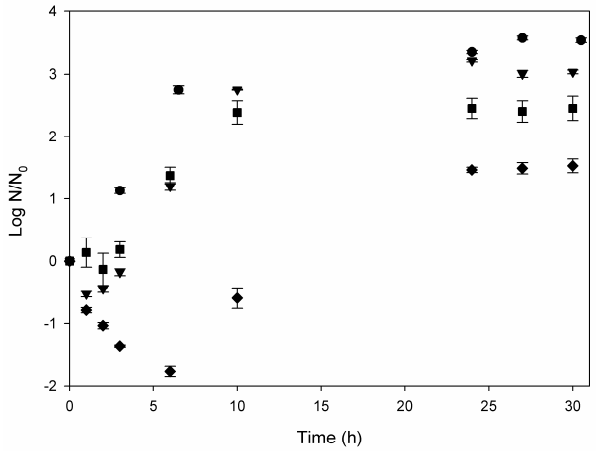
In the past two decades or so, Europe has seen a dramatic rise in the concern amongst citizens about the effects of industrialisation of food production on health. For this reason, there is an increasing interest in "green" food products, that is, with fewer synthetic additives but increased safety, quality and shelf-life; which has led to an interest in the use of natural ingredients with antimicrobial activity as aids in food preservation.

The antimicrobial activity of flavonol-rich cocoa powder against *Bacillus cereus* spores was valuated obtaining growth curves in reference media at different concentrations of cocoa powder (0, 1.5, 2.5 and 5%) and different temperatures (7, 20 and 32 ºC). The results indicated that flavonol-rich cocoa powder had a bactericidal effect at high concentrations and only bacteriostatic at lower concentrations. Growth curves of *B. cereus* were fitted to the modified Gompertz equation to obtain kinetic parameters. A significant decrease in the specific growth rate ($p<0.001$) was observed when increasing the flavonol-rich cocoa powder concentration. Regarding the elongation of the lag phase, the effect of increasing the flavonol-rich cocoa powder concentration was significant at 32ºC.

This study confirms the potential for flavonol-rich cocoa powder to prevent *B. cereus* outgrowths, which could be considered as an additional control measure in the case of cold chain break, and to reduce the growth rate of *B. cereus* in beverages containing cocoa.

Keywords antimicrobial activity, cocoa powder, microbial kinetics, growth rate

FIGURE 1. Growth of *B. cereus* spores at 32 ºC in BHI supplemented with various concentrations of flavonoid-enriched cocoa powder. 0% (●), 1.5% (▼), 2.5% (■), and 5% (◆).



Effects of *Drynaria quercifolia* (Linn.) J. Smith. rhizome extracts against enteropathogenic *Escherichia coli* (EPEC): *In vitro* and *in vivo* studies

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The present study was designed to evaluate *in vitro* and *in vivo* antibacterial effects of petroleum ether (PE), chloroform, ethyl acetate, methanol and aqueous extracts of the rhizome of *Drynaria quercifolia* (Linn.) J. Smith (Polypodiaceae) against enteropathogenic *Escherichia coli* (EPEC). Among the extracts analyzed, PE extract exerted potential growth inhibitory activity against EPEC with inhibition value of 18.9 mm at the concentration of 1000 µg/ml. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of PE extract were 500 µg/ml and 400 µg/ml respectively. Further, the PE extract was subjected to *in vivo* antibacterial activity to study the effect of *D. quercifolia* in *E. coli* infected mice. The results of the *in vivo* study showed that treatment with the PE extract of *D. quercifolia* possessed remarkable effects on mortality and on the number of viable EPEC recovered from feces of mice. Histopathological observation of in the liver, spleen and the small intestine showed restoration of normalcy in PE treated mice. The present finding is hence highly encouraging in recognizing *D. quercifolia*, a medicinal fern, as a potent source of antibacterial agents. Our results may also stimulate the search for antibacterial agents which may be useful for pharmaceutical industries.

Keywords *Drynaria quercifolia* (Linn.) J. Smith; enteropathogenic *Escherichia coli*; antibacterial activity

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Effects of plant extract and MAP on fresh crimson snapper (*Lutjanus erythropterus*) fillets coated with sauce during chilled storage

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This study evaluated the effect of oregano essential oil as a natural preservative in combination with sauces made from Australian native plants with antimicrobial efficacy; lemon myrtle, Tasmannia pepper leaf and kakadu plum. Treated fillets were stored in modified atmosphere packaging (MAP 60% CO_2/40%N_2) while shelf life studies were conducted.

Oregano essential oil and water extracts of lemon myrtle, Tasmannia pepper leaf and kakadu plum were screened for antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* using a microtitre assay. Crimson snapper (*Lutjanus erythropterus)* fish fillets were dipped in an aqueous solution of 0.1% v/v oregano essential oil and 1.5% v/v glycerol. Two sauces were prepared using the following combinations of Australian native plants and other herbs and spices: Sauce A - lemon myrtle flakes (0.15% w/w) and kakadu plum powder (0.5% w/w) and Sauce B – ground lemon myrtle (0.2% w/w), ground Tasmanian pepper leaf powder (0.25% w/w) and kakadu plum powder (0.5% w/w). Another two sauces were prepared with traditional chemical preservatives in place of the native ingredients. Potassium sorbate (0.665%) and sodium benzoate (0.059%) were used at permitted levels as given in Standard 1.3.1 of the Australia New Zealand Food Standard Code .The base mix for the sauces that consisted of xanthan gum, starch and water was taken as the control. The sauces were stored at 4°C and 25°C for 30 days and challenged with the following microorganisms: *Staphylococcus aureus* and *Escherichia coli* (10^5cfu/g), *Saccharomyces cerevisiae* (10^5cfu/g) and *Aspergillus niger* (10^5cfu/g). The fish fillets were dipped in oregano essential oil and then coated with sauces A and B and packaged in modified atmosphere and stored at 4°C for 12 days. Results for the antimicrobial screening of the plants extracts revealed complete inhibition of *Staphylococcus aureus* and *Escherichia coli* at 0.078% of oregano essential oil. Lemon myrtle, Tasmannia pepper leaf and kakadu plum ethanol extracts were completely inhibited at 2.1, 3.2 and 3.9 mg /Gallic acid equivalents(GAE) /g respectively. Sauces incorporating native plant ingredients or chemical preservatives had a total bacterial count <10 cfu/ml and yeast and mold counts < 100 cfu/ml at the end of 30 days when stored at 4ºC and 25ºC. No growth of *Staphylococcus aureus* and *Escherichia coli* was observed in any of the sauces when challenged with the given bacteria. Growth of *Aspergillus niger* and *Saccharomyces cerevisiae* was detected in the control after 7 days while there was no growth observed in the sauces containing native ingredients and chemical preservatives. There was a significant drop > log 1 cfu/g in total viable count and anaerobe count after the oregano essential oil dip (figure 1). This system of oregano dip in combination with sauces made with antimicrobial plant extracts and MAP achieved a shelf life extension of 6 days more than reported in previous studies (figure 2), before the total counts were above log 7 cfu/g. The combination of plant extracts and MAP packaging used for shelf life extension of fish fillets without the use of chemical sanitisers or preservatives demonstrates the potential of natural antimicrobials, such as native plant products, as natural preservatives in multifactorial preservation models incorporating packaging and low temperature storage. The Australian native food industry with its diverse and rich flora has an enormous potential to contribute to this market of natural ingredients. An extensive range of native foods is available in Australia that possesses significant health promoting and other functional properties in addition to their unique flavour profiles.

Keywords plant extracts, shelf-life extension, fish, MAP

Figure 1

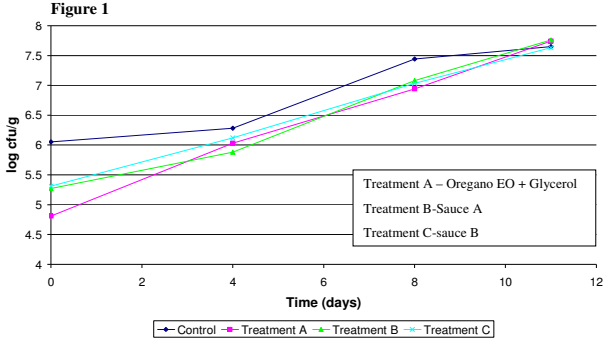
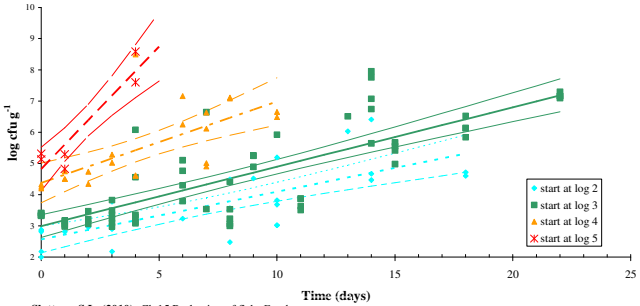


Figure 2



Slattery, S.L. (2010): Ch 15 Packaging of fish, *Food packaging: principles and practice*, Marcel Dekker publishers New York

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Epigallocathechin gallate modulates virulence factors in the pathogenic bacterium *Staphylococcus aureus*

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Exposure of Staphylococcus aureus to sublethal doses of epigallocathechin gallate (EGCG) led to adaptation to EGCG that was accompanied by thickening of the cell walls, increased resistance towards antibiotics targeting the cell wall and increased heat resistance (Bikels-Goshen et al., Int. J. Food Microbiol. 138:26-31, 2010). Extended characterization of morphological and molecular alterations upon adaptation to EGCG is the subject of this report. Mats assay revealed that adaptation to EGCG led to significant increase in the hydrophobicity and acidity of the bacterial surface. In addition, adapted cells were more resistant to the activity of the autolytic enzyme lysostaphin than their control counterparts. Growth in the presence of EGCG was also accompanied by elevated production (6 fold) of biofilm, a major virulence factor. A major hallmark of Staphylococcus is its resistance to oxidation either in the environment or inside phagosome of white blood cells. Exposure to UV light revealed a 1 log suppressed killing effect on of EGCG- adapted cells. Reverse transcription quantitative PCR (RT-qPCR) was used to quantify the transcription level of three genes encoding cell wall biosynthesis (murA, murF and pbp2) and the cell wall biosynthesis positive regulatory gene (vraR). RT-qPCR analysis exhibited elevated expression ratios in all tested genes. The most pronounced and rapid increase was obtained in the expression of vraR transcript with 25- fold increase after 5 min followed by 95 fold increase after15 min of exposure to EGCG. Of the genes encoding cell wall biosynthesis, the most pronounced expression ratios were obtained with murF with 5- fold increase after 5 min exposure to EGCG. These findings raise concerns over the potential use of EGCG in therapy and as a food additive in that it may contribute to elevated pathogenicity and to enhancement of microbial resistance mechanisms.

Keywords Epigallocathechin gallate, virulence, *Staphylococcus aureus*

Essential oils formulated in aqueous emulsions as biocides in livestock and agriculture applications

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Essential oils extracted from certain aromatic plants and their derivates have long been reputed to present antimicrobial properties and repellent activity against insects. Recent investigations confirm the fumigant and insecticidal of some essential oils against a number of economically important insect and mite pests. Regarding livestock applications, essential oils have a stimulating effect on animal digestive systems and have received considerable attention as replacements for antibiotic growth promotants. Besides, the antibacterial and anticoccidial effects of essential oils reduced the total E. Coli in the intestines of animals.

Essential oils typically are volatile and they rapidly evaporate from surfaces. It is thus desirable to formulate them in a way that allows protecting the oil from high temperature, oxidation and UV light, minimizes the evaporation, allows a selective release and increases the shelf life of the oil. For this purpose, the formulation of the essential oils from lavandin and rosemary in oil-in-water (O/W) emulsions has been studied in this work.

The choice of the surfactant used to stabilize the emulsion is crucial since it must fulfil several requirements: besides being able to stabilize an emulsion with adequate physical properties, for agricultural and food industry applications the surfactant must be biodegradable and non-toxic. For this reason, it has been decided to use biopolymers as surfactants, in particular n-octenyl succinic anhydride (OSA)-modified starches. Moreover, when these starches are used as surfactants, the emulsification process can be coupled with a precipitation process performed by spray-drying, freeze-drying or supercritical precipitation technologies, in which the OSA-modified starch that was used as surfactant in the emulsification step can perform the function of carrier material, thus allowing to obtain a more functional product which can prevent oxidative deterioration and can provide controlled release characteristics.

Oil-in-water emulsions from lavandin and rosemary essential oils were prepared in a rotor-stator machine. Previously, the required hydrophilic-lipophilic balance of each oil was determined using droplet size analysis with emulsifier blends of non-ioninc surfactants (span20; HLB=8.6 and tween20; HLB = 16.7) of varying HLB values. The main variables of the process time, stirring frecuency, surfactant concentration and oil fraction were varied in order to optimise it. The quality of the obtained emulsions was analysed through droplet size distribution measurements and stability tests. Furthermore, antimicrobial activity was tested in vitro againts gram-positive bacteria (staphylococcus aureus) and gram-negative (Escherichia coli).

Keywords lavandin oil; rosemary oil, emulsion, required HLB, antibacterial test,

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Evaluation of Antibacterial Activity of *Sasa borealis* Extracts

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We investigated the antibacterial effects of Sasa borealis extracts against eight bacteria (*Psedomonas aerginosa, Salmonella choleraesuis, Serratia marcescens, Vibrio vulnificus, Bacillus cereus, Bacillus subtilis, Listeria monocytogenes and Staphylococcus aureus*) using broth microdillution assay. Using survival curves the kinetics of bacterial inactivation on extracts exposure was followed 24h and in this way the MIC (minimum inhibitory concentration) values determined by broth microdillution assay were confirmed as the concentrations of extracts that inhibited bacterial growth. *Sasa borealis* extracts showed antibacterial activities against all tested bacteria. MIC of extract of *Sasa borealis* were determined 12.5 mg/mL against *Salmonella choleraesuis*. n-Hexane and chloroform layer of extract of *Sasa borealis* have strong antibacterial activities. MIC of n-Hexane and chloroform layer were deterined 3.13-6.25 mg/mL and 6.25-12.5 mg/mL.

These results suggest *Sasa borealis* extracts effectively inhibit bacterial growth and are useful as antibacterial agents.

Evaluation of Antimicrobial Activity of Seaweed *Spirogyra sp.* from Algeria

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In the aquatic eco system, algae are directly exposed and susceptible to ambient micro organisms such as bacteria and fungi. This work was realized in order to evaluate the antibacterial activity of acetone, ethanol and hexane extracts of fresh water microalgae specie of *Spirogyra sp.* from river El Meah, M'sila, in vitro against three Gram-negative bacteria (*Esherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*), two Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) and a yeast (*Candida albicans*) using disc diffusion method.

Acetone was the best solution for extraction of antimicrobial substances. Results of biochemical analysis of this micro algae show that *Spirogyra sp.* contains (5% proteins, 32,5% soluble sugar, 15,84% lipids, 15,6% phenol acids). Bacteriologic and biochemical tests reveal the presence of a remarkable antibacterial activity of this alga.

Keywords: Oued El Mellah, M'sila, *Spirogyra sp*, antimicrobial activity, biochemical composition.

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Evaluation of Antimicrobial activity of seeds of *Brassica juncea* and *Brassica nigra*

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Brassica juncea and *Brassica nigra* seeds are the key ingredients of the Indian food which enhance flavour of food. These seeds are also found to have medicinal properties. Traditionally they are used for treating lung congestion, inflammation, bronchitis and as a digestive aid. In present study, the antimicrobial activity of various extracts of *Brassica juncea* seeds and *Brassica nigra* seeds was evaluated against a number of organisms such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella flexneri, Salmonella typhi, Vibrio cholera* and *Candida albicans* by agar diffusion method. Ciprofloxacin and Fluconazole were used as standard antimicrobial agents. The antimicrobial activity was expressed in terms of zone of inhibition. Aqueous, methanolic, hydroalcoholic, ethylacetate and petroleum ether extracts of seeds were prepared by cold maceration method. Phytochemical screening of extracts indicated the presence of carbohydrates, proteins, saponins, alkaloids, flavonoids, steroids, tannins and phenolic compounds. Aqueous, methanolic and hydroalcoholic exhibited significant antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Phenolic compounds and tannins might be responsible for antimicrobial activity of mustard seeds. The study reveals that *Brassica juncea* and *Brassica nigra* seeds can be used as potential source of antimicrobial agent in the treatment of infectious diseases, mainly of gastro intestinal tract, confirming the traditional claim.

Evaluation of Fungistatic activity of rice varieties against phytophathogenic fungi

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This study evaluated the *in vitro* antifungal activity of aqueous, methanol and n-hexane shoot extracts (0, 1, 3 and 5%) of three rice varieties (Basmati-385, Basmati-386 and Basmati Super) against two phytopathogenic fungi [*Ascochyta rabiei* (Pass.) Lab. and *Macrophomina phaseolina* (Tassi) G. Goid]. Aqueous and n-hexane extracts significantly suppressed the *in vitro* growth of *M. phaseolina.* The aqueous and n-hexane extracts of different rice varieties caused 21–52% and 18–60% reduction in growth of *M. phaseolina,* respectively. However the effect of methanol extracts was nonsignificant. *A. rabiei* proved less susceptible to these extracts as compared to *M. phaseolina.* Only 1% aqueous extracts of Basmati 385 and Basmati 386 significantly declined the growth of this fungus, while, all other extracts either had insignificant effect or stimulated the fungal growth.

Key words: Antifungal, *Ascochyta rabiei,* genotypes*, Macrophomina phaseolina*, phytopathogens, rice.

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Evaluation of *in vitro* and *in vivo* antibacterial and antifungal activity of "Camelyn M"

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The minimum inhibitory concentration (MIC) of "Camelyn M" active compound obtained from special sort of honey in one of region of Georgia, was determined both by agar and broth dilution methods against some of strains of bacteria and fungy. The antibacterial action of "Camelyn M" was further tested in animal models. "Camelyn M" was seen to possess powerful inhibitory action (0,012-0,150 µg/mL) against most test bacteria in *in vitro* studies. *In vivo* studies showed that the drug offered significant protection ($p<0.001$) to mice challenged with a virulent bacterium.

"Camelyn M" exhibited potent in vitro activities against fluconazole-resistant strains of *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei*, with MICs at which 90% of isolates were inhibited of 0.012 µg/ml, respectively.

Evaluation of leguminous lectins activities against bacterial biofilm formation

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Biofilms are composed by microbial cells that are irreversibly associated with a surface and enclosed in a matrix of polymeric material. Lectins are sugar binding proteins of non immune origin that agglutinate cells and / or precipitate glycoconjugate molecules. Due to their capacity to bind and recognize specific carbohydrates, lectins can be a potent tool in biofilm studies. The search for potential phytochemicals as anti-biofilm agents has become an active area of research, and these proteins can bind to the bacteria or prevent the interaction with the surface and consequently decrease biofilm formation.

Thus, the present work aims to evaluate in vitro the antibacterial activity of plant lectinsfrom Canavalia genus against a panel of bacteria of medical relevance, and to inspect their capacity to interfere on the initial adhesion events and biofilm formation.

The assays were carried out using different concentrations of leguminous lectins, isolated from *Canavalia ensiformis* (ConA), *C. maritima* (ConM) and *C. boliviana* (ConBol). The effect of lectins was tested on *Klebsiella oxytoca* ATCC13182, *Pseudomonas aeruginosa* ATCC10145, *Staphylococcus epidermidis* CECT231 and *Staphylococcus aureus*. The bacterial planktonic growth in the presence of the lectins was determined trough absorbance measurement at 640 nm. Adhesion and biofilm assays were performed in polystyrene plates, and chalenged with the three lectins. The biomass accumulated was quantified using crystal violet staining.

The results showed that ConA emerged as the most promising lectin since it clearly reduced the bacterial plankctonic growth, specially of the Gram+ strains, with MIC values ranging between 30 and 125 µg/mL. ConA also disturbed the initial adhesion events of all bacteria and disturbed the biofilm formation ability of the *Staphylococcus* species for all the concentrations tested. Concerning Gram- bacteria, its biofilm formation ability was only prejudiced with higher concentrations of the lectin. Therefore, the results seem to highlight that the antimicrobial activity of ConA was more noticeable in the disturbance of bacterial adhesion and biofilm formation than impairing planktonic growth.

In conclusion, our results show that lectins, an important class of natural products, possess promising antibiofilm activity, suggesting that they may have therapeutic potential for the pharmacological treatment of biofilm-associated infections.

Keywords Leguminous lectins, Antimicrobial natural products, Biofilm-associated infections control

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Flavonoid and flavonoidglycosides from four plants from hoggar recion south of Algeria

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These four plants distributed on three families. *Apiaceae, Asclepiadaceae,* and *Lamiaceae: Pituranthos chloranthus* , its trivial name is zaza, [1] it is endemic in north Africa [2] the local people eat the fresh stems and the roots, they decoct the flowering branches in water to get a sweet drinks. *Solenostemma argel*, its trivial name is *argel*, the local people used it in treatment of numbers of diseases , they break the branches to suck a bitter solution to treat the coughs, they use this solution for cleaning the eyes [2, 3] . *Marrubium deserti*, this species known as tahrar or aberkako, it is endemic in the sahara[1], the local folk used this plant to treat many diseases such as diabetes, Typhoid, malaria, headaches, and others**.** *Ballota hirsute,* known as Afrgagy[3] and Afis.

So the four plants were subjected to extraction to get the following result: To the best of our knowledge this is the first time that *Pituranthos chloranthus*, is subjected to phytochemical studies, thus, six flavonoids were identified for the first time, in which four of them are new for the genus. From *Solenostemma argel*, four flavonoids were identified; one of them is found for the first time in the genus. Also, this is the first time that Marrubium *deserti*, is subjected to phytochemical studies, so, six flavonoids were identified for the first time in which three of them have not been reported in the genus. Finally, from *Ballota hirsute*, seven flavonoids were identified, four of them are new in this species and one is new for the genus.

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Flax seedcake extract as a product with wide spectrum of antimicrobial activity.

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Nowadays the great problem of medicine are chronic ,non-healing wounds: Infections that very often develop in long- lasting wounds remain a major problem despite decades of advances in antibiotics and antiseptics. The worldwide increase in multidrug resistance in pathogenic bacteria has led to an increasing need for topical antimicrobial products that can be applied to potentially contaminated wounds. Many of the products are admittedly highly cytotoxic toward microbial cells, but unfortunately their cytotoxicity can also interfere with the human tissue. Searching for product, which can be simultaneously effective as an unselective antibiotic useful in case of multibacterial and fungal infections, and also do not indicate cytotoxic activity on human cells; become great problem of modern bio-medical sciences.

Preliminary experiments performed on seedcake extract from genetically engineered flax plant indicate unselective antibacterial and antifungal activity of this product. This material derived from transgenic flax plants which were obtained by plant transformation using three genes coding :chalcone synthase (*CHS*), chalcone isomerase (*CHI*) and dihydroflavonol reductase (*DFR*) ,all controlling the synthesis of antioxidative compounds from phenylpropanoid pathway . Such modification resulted in accumulation of phenolic acids in fibres, polyunsaturated fatty acids in oil and lignans in seedcake.[1] Alkali hydrolised seedcake extract is a rich source of strong antioxidant metabolites as: cumaric acid, ferulic acid, sinapic acid, SDG (flax lignan). Antimicrobial activity of this product was tested on four bacterial species, normally used as a model for analysis of antibiotic resistance : *Pseudomonas aeurginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 11229*, Staphylococcus aureus* ATCC 29213 and ATCC6538*, Escherichia coli* ATCC 25922 and ATCC 11229. Our product indicates strong germicidal activity for all this species. Similar fungicidal effect we observe for analyzed fungal species: *Candida albicans* 10231*, Candida krusei* 264 *, Cryptococus neoformans* 2110. What is important, this product does not indicate any negative effect on growth and morphology of Balb/3T3 cells in cytotoxic assay [2].

Such observation leads as postulate, that product based on seedcake extract obtained from our transgenic flax plants will be very good candidate for unselective antimicrobial mixture. To our best knowledge this is the first report describing the potential of product from genetically engineered flax for antimicrobial application.

Keywords flax; transgenic plants, lignan,antimicrobial activity.

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Free radical scavenging and antibacterial activity of crude extracts from selected plants of medicinal value used in South Africa

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Bioautography, anti oxidant capacity of 1, 1-diphenyl-2-picryl-hydrazyl radical (DPPH) and minimum inhibitory concentration (MIC) assays were carried out on plant crude extracts. Common pathogenic bacteria, *Staphylococcus aures*, ATCC 29213 and *Escherichia coli*, ATCC25922 were used in bioautography broth sprays. *P. sandersonii* extract of methanol (04M) and acetone (04A) showed that their polar components were of highest activity where as with those of *A. amatymbica,* it is the non polar components which showed high activity against both bacteria. *G. perpensa,* (01); *P. prunelloideds,* (02); *E. autumnalis* and *B. setifera,* (09) showed very weak activity. For the MIC assay, *P. sandersonii* illustrated the highest anti bacterial activity against both bacteria used because it showed the lowest MIC of 0,156-0.132 mg/ml when compared to *A. amatymbica* (0.6525 mg/ml).The (DPPH) radical scavenging ability of *P. sandersonii* was highest because it had EC 50 of 0.003916, which is comparable to the reducing capacity of trolox EC50 = 0.003175 which was used as a standard. Acetone extracts showed more components on vanillin sprayed TLC plate indicating that it is a better extractant than methanol.

Keywords: Bioautography; Antioxidant, Minimum inhibitory concentration (MIC); Bacteria; DPPH

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Fungitoxic Activity of and Organic Solvent Extracts of Tagetes Erectus on Phytopathovenic Fungus-*Ascochyta Rabiei*

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The *in vitro* fungitoxic potential of *Tagetes erectus* L. was scrutinized against *Ascochyta rabiei,* the causal agent of chickpea blight disease. The pathogen was exposed to various concentrations (1, 2, 3 and 4% w/v) of aqueous and methanol extracts of flower and shoot of *T. erectus* using food poisoning technique. All the employed concentrations of both flower and shoot extracts significantly suppressed the growth of target fungal pathogen. There was 4-35% and 55-73% reduction in colony diameter of *A. rabiei* due to different concentrations of aqueous flower and shoot extracts of *T. erectus* and 12-50% and 4-42% due to different concentrations of methanolic flower and shoot extracts of *T. erectus,* respectively.

Key words: *Tagetes erectus*, fungitoxic activity, *Ascochyta rabiei,* aqueous and organic solvent extracts.

Genetic Evidence for Inhibition of Bacterial Division Protein FtsZ by Berberine

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Berberine is a plant alkaloid that is widely used as an anti-infective in traditional medicine. *Escherichia coli* exposed to berberine form filaments, suggesting an antibacterial mechanism that involves inhibition of cell division. Berberine is a DNA ligand and may induce filamentation through induction of the SOS response. Also, there is biochemical evidence for berberine inhibition of the cell division protein FtsZ. Here we aimed to assess possible berberine mechanism(s) of action in growing bacteria using genetics tools.

First, we tested whether berberine inhibits bacterial growth through DNA damage and induction of the SOS response. The SOS response induced by berberine was much lower compared to that induced by mitomycin C in an SOS response reporter strain. Also, cell filamentation was observed in an SOS-negative *E. coli* strain (Fig. 1). To test whether berberine inhibits FtsZ, we assessed its effects on formation of the cell division Z-rings, and observed a dramatic reduction in Z-rings in the presence of berberine (Fig. 2). We next used two different strategies for RNA silencing of *ftsZ* and both resulted in sensitisation of bacteria to berberine, visible as a drop in the Minimum Inhibitory Concentration (MIC, Fig. 3). Furthermore, Fractional Inhibitory Concentration Indices (FICIs) showed high level of synergy between *ftsZ* silencing and berberine treatment (FICI values of 0.23 and 0.25 for peptide nucleic acid- and expressed antisense RNA-based silencing of *ftsZ*, respectively). Finally, over-expression of *ftsZ* led to a mild rescue effect in berberine-treated cells (Fig. 4).

The results argue against DNA binding as the primary mechanism of action of berberine and support the hypothesis that the antibacterial properties of berberine are due inhibition of the cell division protein FtsZ. In addition, the genetic approach used here provides a means to rapidly test the activity of other putative FtsZ inhibitors.

Keywords berberine; FtsZ, silencing, mechanism of action, SOS response

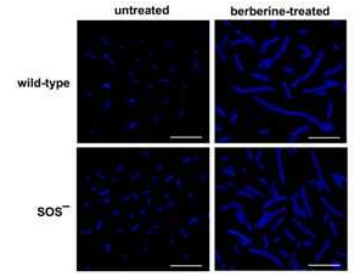

Figure 1.

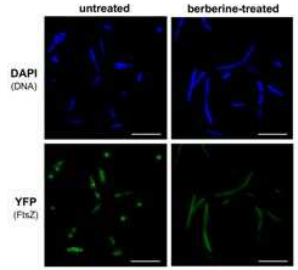

Figure 2.

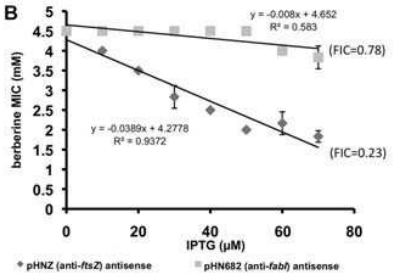

Figure 3.

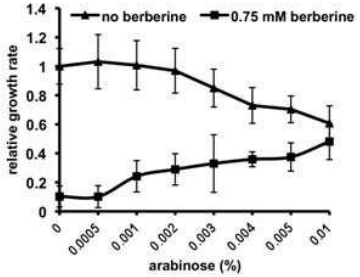

Figure 4.

Geraniol, a compound that restores activities against Multidrug-resistant Isolates from gram-negative species: Improved solubility and identification of structural features involved in biological activity.

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The constant use of antibiotics in the hospital environment has selected bacterial populations that are resistant to many antibiotics. In particular, *Enterobacter aerogenes*, a commensal Gram-negative bacterium of human intestinal flora, has rapidly emerged as an important nosocomial pathogen with an increasing frequency of isolates resistant to many antibiotics and antiseptics.

Several mechanisms contribute to the phenotype of multidrug resistance (MDR) of the bacteria and among them, efflux systems that allow efficient transport of antibiotics. Many gram-negative bacteria share the tripartite efflux pump acrAB. An important medical challenge is to identify compounds capable of circumventing the MDR by the inhibition of efflux systems. To date, the vast majority of the efflux-pump-inhibitors (EPI) described so far are active against Gram-positive bacteria.

The study to be described demonstrates that the geraniol significantly increases the efficacy of chloramphenicol on the *Enterobacter aerogenes* MDR. Moreover, the use of structural analogues of that compound allowed us to identify important features that may account for the activity and the solubility. This opens a new field in search for therapeutic agents capable of restoring the efficacy of currently used antibiotics.

Keywords: Multidrug resistance, AcrAB, Geraniol, *Enterobacter aerogenes*, Efflux pump inhibitors.

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Identification of Antibacterial compounds from the essential oil of seven Indian medicinal plants

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The present paper deals with the isolation of 26 antibacterial compounds from the essential oil of seven medicinal plants. The selected plants were *Piper betle* L., *Sphaeranthus indicus* L., *Tagetes erecta* L., *Clerodendron phlomidis* Linn. f. Suppl., *Coleus amboinicus* Lour., *Premna integrifiolia* Linn. and *Blumea membrancea* DC. The structural details revealed through GC-MS and library comparison.

The essential oils isolated through steam distillation and evaluated in 30 µl/well concentration through Agar well diffusion test. The selected eight bacterial strains were three Gram positive *Bacillus subtilis* (+), *Bacillius cereus* (+), *Staphylococcus aurus* (+) and five Gram negative *Pseudomonas aeroginosa* (-), *Pseudomonas putida* (-), *Salmonella typhi* (-) (Pathogenic strain), *Salmonella paratyphi* (-) (Pathogenic strain) and *Escherichia coli* (-).

The five plants have shown the zone of inhibition ranged between 15mm to 24mm and the value of Minimum Inhibitory Concentration (MIC) is ≤6.25 µl/ml are consider as the most active among all seven species. Those five plants are *Coleus amboinicus*, *Premna integrifiolia, Blumea membrancea, Sphaeranthus indicus* and *Tagetes erecta*.

Key Words: Indian medicinal plants, antibacterial aromatic compounds

In Vitro Anti-*Helicobacter pylori* and Urease inhibitory activities of Resveratrol

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Helicobacter pylori (H. pylori) inhibit the formation of the mucus that covers the surface of the stomach and has been implicated in the aetiology of several gastric pathologies, including chronic gastritis, peptic ulcer, gastric cancer and lymphoma. Recently, it has been found that most adults (approximately two thirds of the world population) are colonized by *H. pylori* [1]. Therefore the number of *H. pylori* carriers undergoing eradication therapy to prevent gastric disorders is increasing.

However, the wide abuse of antibiotics by the modern world has raised a new problem in medicine, namely antibiotic resistance. So, the search of novel chemical compounds with activity against *H. pylori* is a challenge for both medical and scientific communities.

Thus, in recent years a growing interest in biologically active compounds, including antioxidants from plants and other natural sources, has been observed, as some epidemiological studies have shown a correlation between seropositivity to *H. pylori* and environmental factors, including diet. Indeed, a low incidence of infection has been associated with the consumption of vegetables, wine and green tea,[2] while the phytoalexin resveratrol (3,4',5-trihydroxistilbene) is thought to possess antimicrobial effects[3], along with antioxidant properties, which are benefic for the prevention of some diseases, such as cancer[4].

The aims of this work are to investigate the antibacterial properties of resveratrol towards different *Helicobacter pylori* strains (two ATCC and fifteen clinical strains), to determine its minimal inhibitory concentration (MIC) and to study the inhibition of urease *Helicobacter pylori* by resveratrol. The antibacterial activity of resveratrol was determined by following the protocols M45-P and M100-S20, both from the Clinical and Laboratory Standards Institute (CLSI). The disk diffusion method was used to determine the sensitivity of resveratrol while the agar dilution method was used to determine the minimal inhibitory concentration (MIC). The results of the disk diffusion assay and agar dilution showed that resveratrol inhibited the growth of *Helicobacter pylori*. Moreover, resveratrol showed MIC values of 0.025-0.1 mg/mL for all *H. pylori* strains. Resveratrol demonstrated inhibition of urease activity which is considered a virulence factor and aggregation of this organism. In this study we have verified that resveratrol had antibacterial activity against all *Helicobacter pylori* tested strains and inhibited one of the most important virulence factors of *Helicobacter pylori.* Nowadays, the use of natural products as antibacterial agents would be a promising area of investigation.

Keywords resveratrol, urease, *Helicobacter pylori*

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In vitro antibacterial activity and antibiotic synergism of Taxifolin7-*O*-α-L-rhamnopyranoside against multidrug-resistant methicillin resistant Staphylococcus aureus (MDR MRSA)

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The aim of the present study was to evaluate the in vitro activity of Taxifolin-7-O-α-L-rhamnopyranoside (TR) alone and synergism of this flavonoid glycoside combined with four conventional antibiotics, i.e. Ampicillin (AMP), Levofloxacin (LEV), Ceftazidime (CAZ) and Azithromycin (AZM), respectively against selected isolates of methicillin-resistant staphylococcus aureus (MRSA). Individual MICs and MBCs were determined by using broth microdilution method following the CLSI guidelines with standard Mueller–Hinton broth and agar. Synergy tests were performed using the chequerboard method and confirmed by time–kill dynamic test. Susceptibility testing of the clinical MRSA isolates (n = 45) demonstrated that the MIC_{50} (range) and MIC_{90} (range) for TR were (mg/L) 32 (16-128) and 64 (16-128), respectively and the order of potency (MIC ranges) was LEV (8-32)> TR (16-128)> AMP (16-258) >CAZ (128-512)> AZM (1000-8000). Of selected ten of the 45 isolates which belonged to SCCmec III genotype, chequerboard method showed that significant synergies were observed for the combinations of TR with CAZ (FICI(fractional inhibitory concentration indices) =0.187-0.375) and LEV (FICI=0.25-0.5), respectively. Synergy (FICI=0.5, 3 isolates) and indifference (FICI=0.625-2, 7 isolates) were observed for the combination of TR with AMP. And the TR/AZM combination showed indifferent effect (FICI=1-1.5). In the time–kill confirmation test, synergy results kept by the TR and CAZ combination (2.186 log10 cfu/ml increase in killing), but the combination between TR and LEV changed to additivity (1.839 log10 cfu/ml increase in killing). Both combinations of TR with AMP (0.548 log10 cfu/ml increase in killing) and CAZ (0.067 log10 cfu/ml growth) showed indifferences (Figure 1). These results demonstrate that Taxifolin-7-O-α-L-rhamnopyranoside enhances the efficacy of Ceftazidime and Levofloxacin in vitro, which have potential for combination therapy among patients infected with MRSA.

Keywords: Taxifolin-7-*O*-α-l-rhamnopyranoside, susceptibility, antibiotics, synergy, MRSA

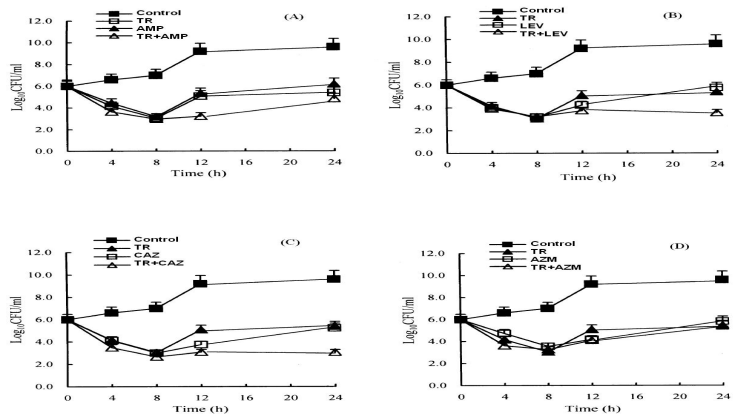

Figure 1. Time–kill curves of the synergistic effect of the combination at 1×MIC (alone) concentration of Taxifolin-7-O-α-L-rhamnopyranoside (TR) and ampicillin (AMP) (**A**), Levofloxacin (LEV) (**B**), Ceftazidime (CAZ) (**C**) and Azithromycin (AZM) (**D**), respectively against MRA 004, a clinical MRSA strains of SCCmec III type.

In Vitro Antibacterial Activity of Extracts of Mangroves *Avicennia marina* (Avicenniaceae) against Enterobacteriaceae Pathogens

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In this present study Antimicrobial activity of aerial parts of Avicennia marina with local name "Hara" eaches its in the Naiband Gulf(IRAN), were evaluated against the pathogens belong to aquatic, human and plant origin, Soxhlet extraction method used to get the corresponding extracts of water, ethanol and methanol.The antimicrobial activities of the organic solvent extracts on the various test microorganisms, including bacteria Enterobacteriaceae (*Pesudomonas arojienes ,cytobacter freundy ,Escherichia coli ,Klebsila oxytoca ,Entrobacter aerogenes, salmonella typhi,Proteus mirabilis,Serratia marescens*) investigated using agar well diffusion. The result of disc-diffusion method was negtive but the assay method and pour plate method which had similar results showed that The Leaves, stem and root of *Avicennia marina* had antimicrobial activity.The leaf of this plant had antimicrobial activity over six species of bacteria out of eleven tested. The leaf extracts with the three solvents water, ethanol and methanol over *P.aeruginosa* showed antimicrobial effect other tested bacteria were sensitive to the extracts of ethanol and methanol the Watery extract was ineffective over them. Stem and root of plant had antimicrobial effects over eight species of bacteria out of eleven tested. The watery, ethanol and methanol extracts of the stem and root of *A.marina* had antimicrobial effects over *P.aruginosa*, *C.freundii*, *S.maresens* other tested bacteria were sensitive to the ethanol and methanol extracts of the stem and root but the aqueous extract was infective over them. The ethanol, methanol and Watery extracts respectively, had more effective antimicrobial effects stem, root and leaf of *A.marina* respectively, had more effective antimicrobial. The antimicrobial effect on gram positive and gram negative tested bacteria was the same. The test of minimum inhibitory concentration (MIC) were done by different extracts of the leaf, tem and root of *A.marina* on sensitive bacteria and results showed that greatest effect was shown by aqueous of *A.marina* root on *P.arunginosa* (MIC=5 mg/lit).

Keywords: Mangroves; Hara,Antimicrobial,Extract,Enterobacteriaceae,Naiband Gulf(Iran)

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In vitro antifungal activity of several essentials oils from aromatic plants of Aragón (NE, Spain)

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Nowadays, the use of these natural antimicrobials for food preservation, is receiving a good deal of attention due to the strong consumer demand for safe and high-quality foods. Therefore, natural substances such as essential oils, safer to consumers and the environment, for the control of postharvest diseases and their effective usage against fungi is gaining popularity.

In this study we have evaluated the antifungal activity of thirteen essential oils against a selection of three strains of the Fusarium genus (*Fusarium verticilloides* CECT 2987, *Fusarium verticilloides* CECT 2982 *and Fusarium graminearum* CECT 2150). The essential oils studied were obtained from plants collected in four different geographical areas of Aragón (north eastern Spain): *Thymus vulgaris local* (L), *Thymus vulgaris French* (F), *Satureja montana*, *Rosmarinus officinalis*, *Lavandin abrialis*, *Lavandin super*, *Lavandin grosso*, *Lavandula latifolia*, *Lavandula angustifolia*, *Lavandula lavandulifolia*, *Hissopus officinalis*, *Salvia officinalis* and *Salvia sclarea*. They all were obtained by hydrodestillation (1 h) from the pulverized aerial parts of the dried plants.

The preliminary *in vitro* antifungal activity was determined by the disc agar diffusion technique. In so doing, cultures of the moulds were grown on PDA slants for 7 days at 25 ºC. Inoculums were prepared by adding about 20 ml of sterile 0.5% Tween 20 in distilled water. Spores were loosened by gentle brushing of the conidiophores with a sterile inoculating loop. The spore suspension was filtered through four layers of sterile cheese cloth to remove mycelial debris and then diluted to a final concentration of 10^6 spores per ml. Then, each plate of PDA was inoculated with 0.1 ml of the spore suspension which was spread on the agar surface and allowed to dry for approximately 2 h. Next, 15 µl of each essential oil were dropped on 6 mm diameter filter paper disks (Whatman No.1) and placed on the agar surface. The plates were incubated at 25ºC for 7 days, after which inhibition zone diameters around each of the disc (diameter of inhibition zone plus diameter of the disc) were measured in millimetres.

The results showed variations in the antifungal properties of plant essential oils. Therefore, the essential oils of *Thymus vulgaris* local, *Thymus vulgaris* French and *Satureja montana* showed an important activity against the three strains studied, by producing a zone diameter of inhibition ≥ 19 mm in all cases. In addition, *Fusarium graminearum* was the most sensitive strain against the essential oils studied, with inhibition zones of 30 mm (*T. vulgaris* local) and whole inhibition (no visible growth of the inoculated moulds) for *Satureja montana* and *Thymus vulgaris* F. On the other hand, no inhibition or just moderate activity was observed for the other essential oils studied against the microorganism tested. However, in those cases in which no inhibition or just moderate activity were observed, it could be seen a lower growth density of the moulds inoculated than in the corresponding negative control (filter paper disk without EO added). Therefore, more precise data about the antifungal activity of these EOs are needed.

Acknowledgements: This research was supported by the Government of Aragón (Spain)-Fondo Social Europeo through Project DGA/Grupo de Investigación Consolidado (Project AO1).

Keywords: essential oils, Aragón (NE, Spain), *Fusarium verticilloides*, *Fusarium graminearum*, antifungal activity.

In vitro Antimicrobial activities of chloroform, hexane and ethanolic extracts of *Citrullus lanatus* var. *citroides* (Wild melon)

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Aim: To test the antimicrobial activities of crude chloroform, hexane and ethanol extracts of leaves, stems, fruits and seeds from *Citrullus lanatus var. citroides* (CL) against bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Proteus vulgaris*) and fungi (*Aspergillus nigar* and *Candida albican*)

Methods and Results: Antimicrobial properties of CL was tested using cup-plate diffusion method and disc diffusion. Analysis of the data revealed that, the chloroform extract of the fruit exhibited the highest antibacterial activities. It showed antibacterial activity against *S. aureus*: 36mm, *B. subtilis*; 38mm, *E.coli*; 37mm, *Pr. valgaris*; 23mm and *P. aerguinosa*; 19mm. The ethanolic extract of the fruit pulp and stem showed the highest antifungal activity on *C. albican* (41mm). A. *nigar* was very sensitive to the chloroform extract of the seed (37mm) and the ethanolic extract of the leaves (37mm). Results were compared concurrently to standard drugs; clotrimazole and gentamicin.

Conclusion: Based on the current findings, it can be concluded that this plant has antimicrobial activity towards certain microorganisms.

Keywords: *Citrullus lanatus var. citroides; antimicrobial; medicinal plants*

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In Vitro Antimicrobial Activity of the Essential Oil of *Erigeron floribundus* (Kunth) Sch. Beep. (Asteraceae)

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The essential oil of *Erigeron floribundus* was screened against ten human pathogenic bacteria and fungi. The oil was found active *against Staphylococcus aureus, Escherichia coli, Candida albicans, Aspergillus niger, Saccharomyces cerevisiae* and *Penicillium chrysogenum* with minimum inhibitory concentration (MIC) of 0.41±0.18, 0.72±0.47, 0.36±0.23, 0.45±0.28, 0.57±0.59 and 0.88±0.63 mg/ml, respectively. The essential oil of *E. floribundus* was found more active against the tested fungal strains.

Keywords Essential oil; *Erigeron floribundus,* Antimicrobial activity

In vitro evaluation of a commercial extract of *Allium sativum* against Methicillin-resistant and Methicillin-sensitive *Staphylococcus aureus* isolates

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Introduction:

Methicillin-resistant *Staphylococcus aureus* (MRSA) included those isolates of *S. aureus* that have become resistant to the antibiotics commonly used to treat ordinary these infections (EFSA, 2009). Most MRSA infections occur in people who have been in hospitals or other health care settings (health care associated MRSA) and therefore MRSA has been recognised as an important cause of hospital-associated infection in humans for several decades. Other type of MRSA infection that has increased during the recent years, occurred among healthy people. These are the community-associated MRSA infections. Recently, an animal reservoir has been demonstrated for certain clonal complex of MRSA. This MRSA clone has been shown to be capable of infecting humans, being considered as an occupational health risk for certain professional groups (e.g. pig holders, veterinarians...).

The antimicrobial properties of several plants extracts, particularly essential oils, have been demonstrated. The activity of extracts of *Allium sativum* against bacteria, yeasts and parasites has been proven (Reuter *et al.,* 1996; Arora *et al.*, 1999; Hunter *et al.*, 2005). In the present study the *in vitro* antimicrobial activity of an allicine extract disposed in a commercial product, Garlicon® (Prebia Feed Extracts SL) and in the two molecules which make up it, PTS (propyl propane thiosulfinate) and PTSO (propyl propane thiosulfonate) have been determined using methicillin-resistant and methicillin-sensitive isolates of *S. aureus.*

Materials and methods:

A total number of 19 isolates of *S. aureus* were included: MRSA (14) and methicillin-sensitive *S. aureus*, MSSA (5). All the isolates were recovered from human cases of infection and most of them were provided by the Instituto de Salud Carlos III, Madrid, Spain. Isolates were grown in TSA agar (Cultimed®) and checked for purity before cultivation in BHI broth (Merck®) during 6 hours at 37ºC under continuous agitation (150 rpm). In order to determine the antimicrobial activity of the tested compounds, a broth microdilution test was performed in 48 wells cell culture plates (Iwaki). Each isolate was tested in one plate that included different concentrations of Garlicon® (5 to 0.83 µl/ml), and PTS (0.63 to 0.063 µl/ml) and PTSO (0.63 to 0.063 µl/ml) in BHI broth and 10^6 CFU of each isolate per well. Positive (BHI + isolate) and negative (BHI) control wells were included in each plate. Plates were incubated during 20 hours at 37ºC with continuous shaking (140 rpm). Minimum bactericide concentration (MBC) and minimum inhibitory concentration (MIC) for each isolate were obtained.

Results and Discussion:

Results are summarized in Table 1. Our results demonstrate the antimicrobial activity of a commercial extract of *Allium sativum* and its compounds against MRSA and MSSA. As expected, activity was higher for PTS and particularly for PTSO than for the commercial product, Garlicon®. No differences were found in the antimicrobial activity between MRSA and MSSA isolates. According to our results, further studies should be performed to confirm the utility of these products as a therapeutic or prophylactic options in MRSA infections in both humans and reservoir animals.

	MIC 90 / MBC 90 (µl/ml)			MIC 50 / MBC 50 (µl/ml)		
	Garlicon®	PTS	PTSO	Garlicon®	PTS	PTSO
MRSA	1.9/2.35	0.4/0.48	0.29/0.39	1.67/2	0.36/0.42	0.13/0.2
MSSA	1.87/2.3	0.38/0.47	0.29/0.38	1.67/2	0.25/0.31	0.13/0.2
Global results	2/2.5	0.42/0.5	0.31/0.42	1.67/2	0.31/0.42	0.13/0.2

Table 1. Minimum inhibitory concentrations (MIC µl/ml) and minimum bactericidal concentrations (MBC µl/ml) 50 and 90 for Garlicon®, PTS and PTSO against Methicillin-resistant and Methicillin-sensitive isolates of *Staphylococcus aureus*.

Keywords MRSA, allicine, *Allium sativum,* antimicrobial activity

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In vitro evaluation of a commercial extract of *Allium sativum* against *Salmonella* isolates of swine origin

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Introduction:

Salmonella enterica is nowadays one of the major pathogens implicated in human food-borne illnesses (EFSA, 2010). Although eggs and poultry meat remain as main sources of *Salmonella*, during the last years pork and pork products have been recognized as the third relevant source of *Salmonella* (EFSA, 2010). According to this, *Salmonella* control programs are been implemented in broiler chickens, layer hens and pigs in different countries including all the steps of the food chain from the primary production, the slaughterhouses, distribution and retail following the "*from stable to table*" approach to food safety as stated in the EU regulations (Zoonoses Directive, 2003/99/EC). On the other hand, the use of antibiotics has been restricted during the latest years in animal production. In this situation, new tools and strategies for the control of *Salmonella* at the farm level are of great interest. The allicine obtained from extracts of *Allium sativum* has demonstrated not only antibacterial but also antifungical and antiparasital activity (Reuter *et al.,* 1996; Arora *et al.*, 1999; Hunter *et al.*, 2005). In the present study, a commercial allicine extract, Garlicon® (Prebia Feed Extracts SL) and the two molecules which make up it, PTS (propyl propane thiosulfinate) and PTSO (propyl propane thiosulfonate) have been evaluated as potential antimicrobial substances in a *in vitro* assay with *Salmonella enterica* isolates from swine.

Methods and materials:

A total number of 61 *Salmonella* isolates obtained from swine faeces and mesenteric lymph nodes, including the most frequent serotypes identified in swine farms from Spain: *S.* Typhimurium (25), *S.* Rissen (10), *S.4,[5],12:i:-* (8), *S*. Derby (7), *S.* Enteritidis (4), *S*. Anatum (3), *S*. Cholerasuis (1), *S*. Newport (1), *S*. London (1) and *S.* Kapemba (1), were tested. All isolates were grown in BHI (Merck) during 6 hours at 37ºC under continuous agitation (150 rpm). A broth microdilution test was performed in 48 wells cell culture plates (Iwaki). Each isolate was tested in one plate that included different concentrations of Garlicon® (5 to 0.83 µl/ml), PTS (0.63 to 0.063 µl/ml) and PTSO (0.63 to 0.063 µl/ml) in BHI broth and 10^6 CFU per well. Positive (BHI + isolate) and negative (BHI) control wells were included in each plate. Plates were incubated during 20 hours at 37ºC with continuous shaking (140 rpm). Minimum bactericide concentration (MBC) and minimum inhibitory concentration (MIC) for each isolate were obtained.

Results and Discussion:

Results are summarized in Table 1. PTSO was the most active molecule against *Salmonella.* A slightly lower activity was seen for PTS while the antimicrobial activity of the commercial product, Garlicon®, was significantly lower as can be expected since this product includes PTS and PTSO (40% of the formulation) and an excipient. A similar antimicrobial activity was found for all serotypes tested. According to this, no differences could be expected while using these products to control swine salmonellosis in farms infected by different *Salmonella* serotypes.

	MIC 90/MBC 90 (µl/ml)			MIC 50/MBC 50 (µl/ml)		
Serotype	Garlicon®	PTS	PTSO	Garlicon®	PTS	PTSO
Typhimurium	2.5/5	0.4/0.5	0.13/0.25	2/2.5	0.25/0.42	0.13/0.25
Rissen	1.25/2.75	0.31/0.63	0.13/0.26	1.25/2.5	0.31/0.5	0.13/0.25
Derby	2.5/5	0.31/0.5	0.13/0.25	2.09/2.5	0.31/0.42	0.13/0.25
O:4[5],12:i:-	1.67/2.5	0.34/0.48	0.13/0.25	1.25/1.67	0.31/0.42	0.13/0.25
Enteritidis	2.25/4.1	0.13/0.25	0.25/0.31	1.46/1.83	0.13/0.25	0.19/0.28
Global results	2.5/5	0.42/0.5	0.13/0.25	1.67/2.5	0.31/0.42	0.13/0.25

Table 1. Minimum inhibitory concentrations (MIC µl/ml) and minimum bactericidal concentrations (MBC µl/ml) 50 and 90 for Garlicon®, PTS and PTSO against different *Salmonella* serotypes isolated from swine.

Keywords Salmonella enterica, allicine, *Allium sativum,* antimicrobial activity

In vitro evaluation of the antimicrobial activity of selected South African honeys and their solvent extracts on Helicobacter pylori isolates.

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The current rising prevalence of antibiotic-resistant *Helicobacter pylori* strains has led to a re-evaluation of the therapeutic use of natural products which are perceived as pure, and without side effects. This has led to the resurgence of interest in honey's therapeutic properties. In this study, two locally produced South African honeys; Champagne Royal Train (CRT) and Honeyleine (HL) were screened for anti-*H. pylori* activity at 10%, 20%, 50% and 75 % (v/v) concentrations by agar well diffusion method. Subsequently, the honeys were extracted with n-hexane, diethyl ether, chloroform and ethyl acetate employed in order of increasing polarity. The solvent extracts were also examined for anti-*H. pylori* activity by the agar well diffusion technique. Clarithromycin was used as positive control. The two most active extracts of each honey were assayed to determine their Minimum Inhibitory Concentrations (MIC_{50}) using broth microdilution method. MICs were recorded by a spectrophotometer at 620nm. Data were analyzed by one-way ANOVA analysis at 0.05 probability level. All honeys showed antibacterial activity at various concentrations against the test isolates, with the strongest activity at 50% concentration, with percentage susceptibilities of test isolates of 56.7(CRT) and 63.3(HL). The positive control recorded percentage susceptibility of 76.7. The most active extracts of CRT and HL honeys were diethyl ether (28/30, 93.3%) and ethyl acetate (22/30, 73.3%) respectively. The other extracts were equally active but lesser antibacterial activity was observed with ethyl acetate extract of CRT (23/30, 76.7%) and chloroform extract of HL (19/30, 63.3%). However, no statistically significant difference ($P>0.05$) was reached comparing zone diameters (mean ± SD) of the extracts of the different honeys (except the chloroform extracts) as well as zone diameters (mean ± SD) of the different extracts to the positive control. The diethyl ether and chloroform extracts of CRT honey and also, the diethyl ether extract of HL honey had MIC_{50} values in the range 0.625-10%v/v. Similarly, the MIC_{50} value of ethyl acetate extract of HL honey was in the range 0.156-10% v/v. However, comparing the MIC_{50} values of all the extracts to amoxicillin(0.001-1.25mg/mL), only the chloroform extract of CRT honey recorded no statistically significant difference ($P>0.05$), indicating this extract to be the most active. In conclusion, these honeys and their solvent extracts contain putative antimicrobial components which when isolated and characterized could serve as leads for the production of novel antimicrobial agents to be employ in the treatment of *H. pylori* infections.

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In vitro photokilling of *Enterococcus faecalis* using the natural compound curcumin in vehicles of alginate and cyclodextrin

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Curcumin, a natural compound in turmeric, has been investigated as a photosensitizer in antimicrobial photodynamic therapy (aPDT). Curcumin in its neutral and most stable state, is almost insoluble in water. In the present study, curcumin was solubilized in hydroxypropyl-β-cyclodextrin (HPβCD) or hydroxypropyl-γ-cyclodextrin (HPγCD) and included in alginate foams or solution. Alginate foams are suitable for wound care applications, and curcumin loaded alginate foams are therefore suggested in aPDT of infected wounds [1].The adsorption/absorption of curcumin from cyclodextrin (CD)-alginate solutions and the phototoxicity of the two curcumin loaded alginate foams in gram positive *Enterococcus faecalis* (*E. faecalis)* were investigated.

METHODS

Curcumin solution:81 µM curcumin solubilized in 3% (w/v) HPγCD and 3% (w/v) sodium alginate was prepared by adding a stock solution of curcumin in ethanol to the CD-alginate solution in phosphate buffer (pH 5).

Foams: Curcumin (0.18%, w/w) was loaded into an alginate foam matrix [1].

Investigation of curcumin adsorption/absorption in *E. faecalis*: Colonies of *E. faecalis* was transferred to the surface of curcumin solubilized in CDs and alginate solution and incubated for 45 min at 37°C. The bacteria were washed twice with PBS (pH 6.1) and transferred to sterile water before examination by fluorescence microscopy with a camera attached (Olympus BX51 and Olympus DP70).

Foam phototoxicity on bacteria: Bacteria were exposed to curcumin loaded foams for 1 h prior to irradiation with blue light (lamp with fluorescent tubes: emission maximum 450 nm; radiant exposure ~10 J/cm^2) prior to incubation for 1 h [1].

RESULTS AND DISCUSSION

Green fluorescence emitted from a curcumin solution containing in *E. faecalis* indicated that curcumin was adsorbed/absorbed to the bacteria (Fig. 1). Curcumin loaded alginate foams in combination with blue light were highly phototoxic towards *E. faecalis* (Fig. 2.).

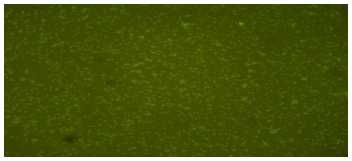

Fig. 1: Fluorescence microscopy image of curcumin (in HPγCD/alginate solution) incubated (45 min) with *E. faecalis* (1000x magnification, excitation filter: 420-480nm).

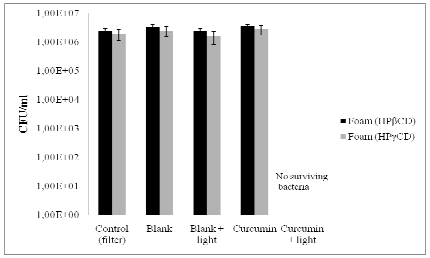

Fig. 2: Phototoxicity of curcumin loaded alginate foams in bacteria expressed as the number of surviving colony-forming units (CFU/ml). Data are means ± SD (n = 9). Blank: alginate foams without curcumin; control: filter discs

CONCLUSION

Curcumin in combination with blue light shows promising phototoxicity towards *E. faecalis*. aPDT of these gram positive bacteria with curcumin solubilized in HPβCD or HPγCD and loaded into alginate foams, resulted in 100% inactivation of viable bacterial cells when combined with 10 J/cm^2 radiant exposure. Bacteria were not affected by curcumin treatment under dark conditions.

Keywords curcumin, E. faecalis, alginate, cyclodextrin, photosensitizer, antimicrobial photodynamic therapy, formulation

1.Hegge, A.B., et al., Formulation and bacterial phototoxicity of curcumin loaded alginate foams for wound treatment applications Studies on curcumin and curcuminoides XLII. Journal of Pharmaceutical Sciences, 2010: DOI 10.1002/jps.22263.

In-vitro Bioactivity of crude acetone and aqueous extracts of the stem bark of *Sclerocarya birrea* (A. rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro) (Anacardiaceae) on clinical isolates of *Helicobacter pylori*

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In an attempt to identify novel sources of cheap starting material for the synthesis of new drugs, the antimicrobial activity of five solvent extracts of the stem bark of *Sclerocarya birrea* was investigated against 30 clinical strains of *H. pylori* and a standard control strain NCTC 11638 by agar well diffusion and micro-broth dilution methods. Metronidazole and amoxicillin were included as positive control antibiotics. The active phytocomponents were detected by TLC and indirect bioautography using ethylacetate/methanol/water (5:2.7:2.5) as eluent. Results were analysed using SPSS version 17.0 and Excel. One way ANOVA test was used to compare activity of extracts and antibiotics and $P<0.05$ considered for statistical significance. All the extracts exhibited anti- *H. pylori* activity (zone diameters of inhibition 0 – 21mm). The acetone and aqueous extracts showed potent anti- *H. pylori* activity with percentage susceptibilities of 70.0% and 73.3% respectively. The lowest MIC_{90} value of 0.06mg/mL was recorded for the acetone extract which did not differ significantly with the MIC_{90} of the aqueous extract (0.16 –2.50mg/mL) and amoxicillin (0.01 – 0.63mg/mL), $P>0.05$, but were significantly different from those of metronidazole (0.16 – 5.0mg/mL), $P<0.05$. Most of the active phytocomponents were located in the acetone crude extract $R_f \leq 0.62$, with more than 90% inhibition. In both extracts, components with lower R_f values exhibited better inhibitory activity. These results demonstrate that the acetone and aqueous extracts of *S. birrea* may contain compounds with therapeutic activity against *H. pylori*.

Key words: Antimicrobial activity; Bioautography; Crude extracts; Drug discovery; *H. pylori*; MIC.

Inactivation of *Escherichia coli* O157:H7 by a synergistic preservation combined process of heat and citral

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During the last few decades, consumers have displayed an increasing tendency to maintain a diet that promotes better health. Consequently, food industry is working to develop new preservation techniques to offer fresh produce that preserves the nutritional and sensorial value while ensuring its stability and security. In this sense, nowadays one of the most common strategies lies in the application of the hurdle theory, which proposes the combination of two or more preservation techniques in order to decrease their impact on food sensory characteristics and improve the lethal efficacy of the treatment at the same time. In accordance with the general interest for natural products, the combination of essential oils with another preservation technology (such as heat, high hydrostatic pressure or pulsed electric fields) at low intensities might accomplish a synergistic lethal effect in the inactivation of emerging pathogens in ready-to-eat food.

Previous work in our research group had showed that sublethal injury caused on the outer membrane of Gram-negative bacteria by mild heat treatments could allow the access of hydrophobic substances (like those present in essential oils) to the cell, while otherwise they would hardly inactivate bacterial cells. Consequently, this investigation was carried out to propose a new combined process of heat and citral to achieve 5 $\log_{10}$ cycles of inactivation of *Escherichia coli* O157:H7 in apple juice. For this purpose, we studied the heat resistance of this pathogen in apple juice and citrate-phosphate buffer of the same pH, as well as the occurrence of sublethal injury in *E. coli* O157:H7 membranes after heat treatments, and the inactivating effect of citral at room temperature. After having determined the treatment conditions, the combined process was assayed in the buffer medium, and was afterwards validated in apple juice.

Heat treatments were performed in a thermo-resistometer or in a thermostatic bath. Survivors and sublethally injured cells were determined by counting the number of colony forming units after incubation of samples onto a non-selective medium and two selective media containing sodium chloride or bile salts.

Our results demonstrated that, at low intensity heat treatments, a protective effect of apple juice was shown: whereas inactivation followed a linear kinetics in citrate-phosphate buffer, when the cells were treated in apple juice survival curves were concave downwards, and a significantly greater time was needed to inactivate 90% of the initial cell population within the range of 54-62°C. Nevertheless, heat resistance of *E. coli* O157:H7 was similar in both media when considering a higher level of inactivation (for example, 3 $\log_{10}$ cycles) at the same temperatures.

As expected, a heat treatment at 54°C for 4 minutes induced sublethal injuries in both cytoplasmic and outer membranes to a great extent, since it caused the inactivation of less than 0.5 $\log_{10}$ cycles of *E. coli* cells but sublethally injured 1 $\log_{10}$ cycle of survivors in the cytoplasmic membrane and more than 3 $\log_{10}$ cycles in the outer membrane.

Although the addition of 200 ppm of citral at room temperature to apple juice or buffer medium containing $3 \cdot 10^7$ CFU/mL did not cause the inactivation of more than 50% of the initial *E. coli* O157:H7 population, when applying a combined process of 200 ppm of citral and heat (54°C) at the same time, more than 5 $\log_{10}$ cycles of inactivation were achieved after 10 minutes. Specifically, the decimal reduction time went from 9.2 min (after the heat treatment) to 1.7 min when citral was added. The same synergistic lethal effect was found after the addition of 18 ppm of citral at an initial contamination level of $3 \cdot 10^4$ CFU/mL at 54°C in apple juice.

Therefore, this study showed the possibility of combining a mild heat treatment with a small quantity of citral in order to control *E. coli* O157:H7 in apple juice and to minimize the possible negative effects of these treatments at higher intensities on food properties.

Acknowledgements: This study was supported by the CICYT (Project AGL 2009-11660). Thanks are also given to Gobierno de Aragón, which provided L. Espina with a grant to carry out this investigation.

Keywords citral; heat treatment; outer membrane; Mafart equation; sublethal injury; *Escherichia coli* O157:H7

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Influence of some natural compounds on freshwater microfoulants

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The antifouling properties of five natural compounds (poly-alkyl pyridinium salts – pAPS, *Ceramium botryocarpum* extract – CBE, zosteric acid – ZA, capsaicin – CS and cinnamaldehyde – CI) were tested against mixed and isolated freshwater biofilm microbial components, in laboratory conditions. These active natural agents can be extracted from marine (*Reniera sarai*, *Zostera marina*, *Ceramium botryocarpum*) or terrestrial (chili pepper, cinnamon) natural sources and for some of them (ZA, CS and CI), the chemical analogues can be synthesized as well. The evaluation of their efficacy in liquid and solid cultural medium, using serial dilution and diffusion method, was performed. The most efficient antibiofouling agents (ABAs) seems to be pAPS and CI at all tested concentrations. ZA exhibited a contrary behavior with respect to the others inhibitors, being less efficient at the highest concentrations. However, most of the ABAs tested are efficient against cyanobacteria rather than algae, and only CI showed a inhibitor effect against fungi as well.

Inhibition of Betalactamase by hydroquinone and its molecular modeling interaction studies

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Beta lactam antibiotics are the most clinically important antimicrobials. The destruction of the antibiotics by the enzyme beta lactamase is the important mechanism of the beta-lactam antibiotic resistance. Resistance to beta lactam antibiotics among target pathogens developed early in the history of their use. Resistance to beta lactams mediated by beta lactamases can be overcome successfully with the use of beta lactamase inhibitors. Combinations of beta lactam and beta lactamase inhibitors have become one of the most successful antibacterial strategies in our global battle against bacterial infections. The combination of beta lactams with beta lactamase inhibitors restores the activity of the beta lactams, allowing their continued clinical use. The synthetic beta lactam inhibitors are now common in use but they have lot of adverse effects and expensive. It is reported here the inhibitory effect of phytochemical, hydroquinone, isolated from the plant *Pongamea pinnate* on beta-lactamase. The compound was found to have synergistic effect with the antibiotic amoxicillin against resistant strain of *Staphylococcus aureus*. The enzyme was purified from the organism and incubated with the compound. An assay showed that the compound can inhibit the enzymatic activity of beta-lactamase. Modeling and molecular docking studies indicated that the compound can fit into the active site of beta-lactamase and can mask the important residue for hydrolysis of beta lactams. Hence the compound can serve as a potential lead compound for the development of effective beta-lactamase inhibitor.

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Investigation of antimicrobial activity of naturally-occurring compounds and traditional herbal medicines

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Animal disease outbreaks and emergency of antimicrobial resistance have placed a great challenge to healthcare systems worldwide. The EU-wide ban on antimicrobial growth promoters (AGPs) in food animal production since 2006 has also created a gap and alternatives are ugently required in the lights of European Commission's current effort on promoting "health animal equals health human". The potential antimicrobial effects of some naturally occurring compounds and crude extracts of traditional herbal medicines were investiged for possible activity against a number of animal pathogens that are of economic importance including *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), porcine reproductive and respiratory syndrome virus (PRRSV), bovine herpes simplex Virus Type 1 (BHV-1) and bovine enterovirus (BEV). Six natural compounds (cinnamaldehyde, cinnamon oil, carvacrol, oregano oil, 2,5-dihydroxybenzaldehyde and 2-hydroxy-5-methoxybenzaldehyde) were found to inhibit the growth of *Map*, while crude extracts of three medicinal plants (*Herba agrimoniae, Rhizoma smilacis chinensis and Sargentodoxa cuneata*) exhibited antiviral effects on all three viruses tested. Prelimary studies on possible mode of action of the anti-Map compounds showed that all compounds caused leakage of phosphate ions to the extracellular environment in a time and concentration dependent manner. None of the compounds caused leakage of ATP to the extracellular environment. An absorbance scan of the supernatant of a *Map* culture incubated with cinnamaldehyde showed that a peak with λ_{max} at ~250 nm appeared after 24 h incubation, which might be indicative of leakage of intracellular constituents, such as proteins or nucleic acids. Results of the study also suggested that the antiviral activities of three plant extracts may be due to the inhibition of replication of virus. Further investigation will be focused on evaluation of potential application of these active compounds and/or extracts in food animals for the control of diseases and food pathogens.

Keywords: animal diseases outbreaks, virus, natural products, medicinal plants, pathogens, AGPs, antimicrobial, resistances.

Investigation of the Antimicrobial Activities of Three Medicinal Plants on the Genus Shigella and Salmonella Causing Diarrhoea in Children

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Diarrhoea, particularly infectious diarrhoea in children less than 5 yrs of age is labeled as the second leading cause of mortality and morbidity throughout the world. This is especially true in developing countries like Ethiopia where there is poor sanitation and overcrowding. Among the leading causes of infectious diarrhoea, *Salmonella* and *Shigella* contributes a lot. Currently the chemotherapeutical treatment of salmonellosis and shigellosis is complicated as a result of drug resistance. Moreover, since the majority of the people who lives in these developing countries have no access for modern treatment, it has made them to look for other alternative therapies such as, the use of medicinal plants.

Ethiopia is one of the well known countries of the world where medicinal plants are used widely. The major objective of this study is to evaluate the antibacterial activity of three medicinal plants (*Gardinea lutea, Olea europaea* subsp. *cuspidata*, *Myrica salisfolia)* against clinical isolates of *Shigella* and *Salmonella* and a control strain *E. coli* ATCC (25922). The minimum inhibitory concentration (MIC) of the three medicinal plant extracts including their semi purified fractions, and modern antibiotics were determined, using the standard agar dilution method (NCCLS). Those fractionated extracts which have shown weak to high antimicrobial activity and the three antibiotics, (Chloramphinicol, Tetracycline and Norfloxacin) have been tested in three replicates. From the three plants of both the crude and semi purified fractionate of *Olea europaea* subsp. *cuspidata* has shown weak activity against both *Shigella* and *Salmonella*. The MIC of *Olea europaea* subsp. *cuspidata* is > 2000µg/ml for both clinical isolates. The other two plants (*Gardinea lutea* and *Myrica salicfolia*) have shown relatively better MIC value, particularly against the clinical isolates of *Shigella* and *Salmonella* species. The range of MIC, where antishigella activity was recorded for both the crude and butanol fraction of *Gardinea lutea* was between 2000µg/ml - 250µg/ml and the range of MIC for both the crude and fractionated extracts *Myrica salisfolia* is greater than or equal 1000µg/ml for both clinical isolates of *Shigella* and *Salmonella*.

As compared to the result of modern antibiotics, it can be suggested that, the plant extracts have shown weak activity with low MIC values. Among the antibiotics, tetracycline, has shown MIC value of >200 µg/ml, for both *Salmonella* and *Shigella*. While chloramphinicol has shown MIC value of <600µg/ml for *Salmonella* isolates and >150µg/ml for *Shigella* isolates. The least MIC value was obtained for norfloxacin with MIC value of > 0.43µg/ml with 100% growth inhibition for *Shigella* and *Salmonella*. Further investigations (purifications) could enhance, especially for the antimicrobial activity of the semi purified butanol fractionates of *Gardinea lutea* and *Myrica salisfolia* which have shown relatively the best activity against the clinical isolates of *Salmonella* and *Shigella*.

Key words: Minimum Inhibitory Concentration (MIC), *Gardinea lutea*, *Olea europaea* subsp. *cuspidata* , *Myrica salisfolia*

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Isolation and identification of antibacterial active compound from *Carum copticum* L.

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Pesticides have made great contribution for quick and effective management of plant diseases and microbial contaminations in several agricultural commodities. In-spite of use of all available means of plant protection, about 1/3 of the yearly harvest of the world is destroyed by pests and loss due to this is expected to be nearly $300 billion per year. Incessant and extensive use of these synthetic pesticides are posing serious problem to the life supporting systems due to their residual toxicity. Effective phytocompounds are expected to be far more advantageous than synthetic pesticides, as they are easily decomposable, not environmental pollutants and posses no residual or phytotoxic properties. Further more it is also true with human pathogenic bacteria. There impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance, along with appearance of undesirable side effects of certain antibiotics.

Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind, especially in the line of medicine and pharmacological. The purpose of this study was to evaluate the antibacterial potential of seven medicinal plants *Acacia nilotica* (L) Del. (Leaf), seeds of *Carum copticum* L., Seeds of *Annona squomosa* L., *Emblica officinalis* Gaert. (Leaf), *Hyptis suaveolens* Poit. (Leaf), *Millingtonia hortensis* L. (Leaf), and fruits of *Pedalium murex* L also tested along with leaves. Different solvent (Petroleum ether, chloroform, ethyl acetate and methanol) extract was obtained using Soxhlet apparatus and tested against important bacteria viz., *Agrobacterium tumefeciens*, *Erwinia carotovora*, *Pseudomonas solanacearum*, *Pseudomonas syringae*, *Xanthomonas campestris*, *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermis Salmonella typhi, Salmonella enterica* sub sp. *enterica, Salmonella paratyphi* A and *Salmonella tyhimurium.* Among the tested plants, petroleum ether extract of *Carum copticum* have shown significant activity followed by methanol extract *Acacia nilotica, Emblica officinalis* and fruits of *Pedalium murex* by well diffusion method when compared to Bacterimycin and Gentamycin. Isolation and purification of the bioactive principle from *Carum copticum* was carried out using column chromatography and Thin Layer Chromatography. The active fraction with R_f 0.38 as shown significant activity. Spectral analyses of active compound were recorded using KBr discs on FT-IR Jasco 4100 infrared spectrophotometer ^{1}H NMR were recorded on Bruker DRX -500 spectrometer at 400 MHZ using d_6-DMSO as solvent and TMS as an internal standard. Results revealed that the compound 2-isopropyl-5-methylphenol was present**.** MIC tests were carried out according to Resazurin Assay and TTC method by using Muller-Hinton Broth. The results showed MIC between 20 to 12.7 µg/ml for the test pathogens. The finding of the present investigation is an important step towards crop protection strategies for bacterial disease management and human disease management.

Key words: antibacterial activity; medicinal plants; *Carum copticum.*

Isolation of toxic peroxidase and lectin from medicinal herbs: potent sources for novel antifungals

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Antifungal proteins and polypeptides have been isolated from diverse groups of organisms, including plants, fungi, bacteria, insects and animals. The mechanisms of action of these proteins are as varied as their sources and include fungal cell wall polymer degradation, membrane channel and pore formation, damage to cellular ribosomes, inhibition of DNA synthesis and inhibition of the cell cycle. The mode of action of many proteins remains unknown and is a subject of active research. The recognized pathogenesis – related proteins was extensively reviewed and currently comprise 17 families of induced proteins. The present paper reports the purification and characterization two non race specific, broad spectrum antifungal proteins from the medicinal herbs, *Acorus calamus* and *Withania somnifera*. The basic protein isolated from the leaves of *A. camalus* (*AcPOX*) had a molecular mass of 32 kDa with temperature stability up to 60^0C. It inhibited the hyphal extension and caused hyphal branching in several phytopathogens. The peptide sequencing revealed its similarity to bacterial induced peroxidase from *Oryza sativa* with 37% sequence coverage. Similarly, a 30kDa acidic chitin binding, non-hemagglutinating antifungal protein was purified from leaves of *Withania somnifera* (*Wsl*). Peptide sequencing revealed its similarity to concanavalin like lectin from *Canavalia ensiformis*. *Wsl* showed 10 times increased antifungal activity when compared to known antifungal lectins like wheat germ agglutinin and concanavalin. This report adds a new dimension to the utilization of the untapped genetic resources for novel peptides.

Keywords Broad spectrum; fungus; hyphal inhibition**;** phytopathogen**;** proteins

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Lichens: A novel source of Antimicrobial agents

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Lichens are symbiotic organisms composed of a fungal partner (mycobiont) in association with one or more photosynthetic partners (photobiont). They are found worldwide covering 8% of the earth surface. They produce characteristic secondary metabolites that are unique with respect to those of higher plants and have a proven track record in providing novel agents possessing manifold biological activities. However lichens from Himalayas region (Uttarakand) of India are still unexplored and not much studied. In this context, lichens namely *Parmelia reticulata* and *Ramalina roesleri* were screened for various biological activities and a bioassay guided approach was followed to isolate compounds responsible for the activity. Biological activities against plant pathogenic fungi (*Pythium ultimum*, *Rhizoctonia solani* and *Alternaria cochlioides*), human pathogenic bacteria (*E.coli*, *Stapylococcus aureus*, *Bacillus subtilis*, and *Streptomyces viridochromogenes* TÜ 57), and human pathogenic fungi (*Candida albicans* and *Mucor miehei*) was carried out with Disk diffusion method. Among various metabolites isolated, Protolichesterinic acid and Usnic acid was found to be most effective against tested pathogenic microbes at a lower concentration 40 µg per disc. Protolichesterinic acid showed excellent activity against bacteria *E.coli*, *Stapylococcus aureus* and fungi *Mucor miehei* whereas Usnic acid showed a broad spectrum activity against all the tested pathogenic fungi and bacteria.

Keywords: Lichens**,** *Parmelia reticulata* , *Ramalina roesleri,* Biological activity.

Medicinal and Antimicrobial effects of *Thymus daenesis* Celak (Avishan)

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Medicinal plants are potential of antimicrobial compounds. The landscape of Iran includes great ecological diversity and has a rich flora which has not been studied for phytochemistry and bioactivity. Lamiaceae is of the most important plant families in which Thymus with about 215 species is a significant genus. *Thymus* species are commonly used as tonic, carminative, digestive, antitussive, expectorant and for the treatment of cold in Iranian traditional medicine. Among the species grown in Iran, *Thymus daenesis* Celak. and *Thymus kotschyanus* are widely used for these purposes. Our study aimed to investigate the inhibitory effect of essence of *Thymus daenensis* Celak on *Escherichia coli* , *Staphylococcus aureus*, *Salmonella para* B, *Bacillus subtilis* and *Micrococcus* spp. Essence of the plant were prepared and their antimicrobial effects were assessed using "bacterial broth dilution methods". Minimal bactericidal concentration (MBC) and minimal inhibition concentration (MIC) were separately measured for essence of the plant. The results showed that essence of the *Thymus daenensis* Celak sub sp had a MIC and MBC of 1/1200 ml on *E.coli* (ATCC-1231) and on *Staphylococcus aureus* (ATCC-1113). It had a MIC and MBC of1/800 ml on *Salmonella para* B. But it did not show any effect on *Bacilus subtilis*(ATCC-1365) and *Micrococcus spp* (ATCC-1170).

Keywords: Disk diffusion, minimal bactericidal concentration, minimal inhibition concentration

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Modelling the growth inhibition of common food spoilage and pathogenic micro-organisms in presence of solvent extract from Irish York cabbage

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Preservatives are required to maintain the quality, extend shelf life, and ensure safety of fresh and processed food products. Although chemical preservatives form an essential part in food preservation, legislation has restricted their use in different foods. Vegetables in the *Brassica* family (Cabbage, Broccoli, Brussels sprouts) are a rich source of a number of bioactive compounds such as flavonoids, glucosinolates and their breakdown products which may have antimicrobial, antioxidant and anticancer properties. The present study investigates the antimicrobial activities of solvent extract from Irish York cabbage, Brocolli and Brussels Sprouts on the growth inhibition of common food spoilage (*Listeria monocytogenes* and *Salmonella abony*) and food pathogenic (*Pseudomonas aeruginosa* and *Enterococcus faecalis*) bacteria. Out of the three vegetables, extracts from York Cabbage showed the best results. Brocolli and Brussels sprouts, at a concentration of 2.8%, showed a weak inhibition in the range of 47-50% and 20-40%, respectively, against the different organisms. The extracts from York cabbage showed a broad spectrum of antimicrobial activity for the different organisms and the activity was found comparable to common synthetic food preservatives such as sodium benzoate and sodium nitrite. Extracts at a concentration of 2.8% showed varying level of inhibition against *Listeria monocytogenes* (100%), *Salmonella abony* (75%), *Pseudomonas aeruginosa* (65%) and *Enterococcus faecalis* (31%). Growth/survival of the micro-organisms in presence of extract was mathematically modelled using Baranyi model equations. The lower concentrations of cabbage extract prolonged the lag phase and reduced both the maximum specific growth rate and final population densities. Thus, the present study brings a new insight into the use of a commonly available vegetable such as York cabbage to provide an innovative measure as a natural antimicrobial agent with potential to enhance food safety.

Keywords: modelling, antimicrobial, cabbage, solvent

Natural antimicrobial agents against the microbiota associated with insoles

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The footwear industry has been one of the greatest changing businesses in recent decades. For this reason, new materials and technologies must be used in terms of comfort, safety and market competition. Recently there has been a growing interest in natural products. Therefore, the use of these new natural agents incorporated in footwear components approaches a new way of innovation.

During this work the analysis of the microbiota associated with pigskin insoles used by different users has been carried out. Subsequently, the effect of natural antimicrobial agents against the microorganisms associated with these insoles was analysed.

The methodology used was based on the PCR and DGGE techniques so as to determine the composition of the community of microorganisms in the different samples and a further identification. DNA samples from different insoles used were extracted and DNA extracted from unused templates served as negative control. PCRs have been made using specific primers for Bacteria and Eukarya on each sample.

Once the amplification products from Bacteria were obtained, microbial composition was analyzed on a DGGE gel. Each of the bands obtained was further amplified again on a second DGGE in order to check the purity and get enough DNA products for advance sequencing. Furthermore, the bands pattern for each user with the software FPQuest was analysed to determine the degree of similarity between them.

Having identified the microorganisms associated to pigskin insoles, the antimicrobial effect of two natural compounds (tea tree oil and lemon extract) were tested using techniques such as measurement of the absorbance of the culture at 600 nm in liquid medium and measurement of halos inhibition by agar diffusion.

In order to carry out the test in liquid medium, firstly Bacillus subtilis and Staphylococcus aureus (identified by DGGE) in LB and nutrient broth were inoculated, respectively. Four tubes for each species were inoculated and it was poured into each of them: almond oil (allegedly innocuous), lemon extract and tea tree oil (antimicrobial agents). Moreover, an oil-free tube was inoculated as negative control. The inocula have grown up at 37 ° C and 200 rpm and after that, the absorbance at 600 nm after 8 and 24 hours of growth was measured.

On the other side, the evaluation of the inhibition halos assay by seeding four medium plates for each type of bacteria was carried out. It was placed on each of the seeded plates a disc of cloth impregnated with the aforementioned conditions in the liquid medium: almond oil, lemon essence, tea tree oil and an oil-free cloth disc (control).

Both natural agents tested showed an inhibitory effect against the growth of the microbial species tested, thus proving their suitability as possible candidates to be used on different parts of footwear.

Keywords footwear industry; natural agents; microbiota; DGGE; antimicrobial

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Oregano and Cinnamon essential oil reduces swarming motility of *Proteus mirabilis*

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In recent years, there has been an increasing interest in Essential Oils due to theirs high antimicrobial properties and their natural origin. Classified as Generally Recognized As Safe (GRAS), Essential Oils have been used in the development of new and interesting alternatives to extend the shelf life of foodstuff. Packaging containing Essential Oils as active agents, have been created to prevent the microbial proliferation in food, increasing their shelf life.

Bacterial motility is a crucial factor in the colonization of natural environment, including food surfaces. *Proteus mirabilis* exhibit flagella-dependent swimming and swarming motility under certain conditions. Swimming is an individual movement of the cell in liquid medium or soft semisolid agar, whereas swarming, consists of a coordinated cellular behaviour leading to a collective movement on semisolid surfaces. Swarming implies changes in bacterial morphology as elongation or hyper flagellation and is correlated with the expression of virulence proteins, including haemolysin, urease and protease.

The aim of this study is to investigate the effects of subinhibitory concentrationes of Oregano and Cinnamon Essential Oil on *P. mirabilis* motility. To this purpose the motility was measured in soft or normal agar and bacterial morphology was studied using optical microscopy and scanning electron microscopy.

The results showed that Oregano and Cinnamon reduce the swarming motility of *P. mirabilis,* but not swimming motility. Essential oils produce a reduction of swarming differentiation, reducing the number of elongated hyperflagellated swarm cells. Thus, oregano and cinnamon prevent the colonization by *P. mirabilis*

Keywords motility, essential oil

Performance of crossbred calves supplemented with Garlic extract

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Thirty six crossbred calves (Holstein cross) of 5 days of age were used to study the effect of garlic extract feeding on their performance up to the age of 2 months (pre-ruminant stage). They were randomly allotted into treatment and control groups (18 numbers in each group). Performance was evaluated by measuring average body weight (BW) gain; feed intake in respect of dry matter (DM), total digestible nutrient (TDN) and crude protein (CP); feed conversion efficiency (FCE) of DM, TDN and CP; fecal score; fecal coliform count and feeding cost. Diets were same for the both the groups. In addition, treatment group received garlic extract supplementation @ 250 mg/kg BW/day/calf. Body weight measured weekly, feed intake measured twice daily, proximate analysis of feeds and fodders analyzed weekly, faecal scores monitored daily and faecal coliform count done weekly. There was significant increase in average body weight gain, feed intake and FCE and significant decrease in severity of scours as measured by faecal score and faecal coliform count in the treatment group compared to the control group ($P<0.01$). Feed cost/kg BW gain was significantly lower in the treatment group compared to control group ($P<0.01$). The results suggest that garlic extract can be supplemented to the calves for better performance.

Keywords: Calf, Pre-ruminant, Garlic extract**,** Body weight gain**,** Faecal score, Faecal coliform count.

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Phytochemical and antibacterial properties of Combretum mucronatum leaf extract

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The cold extraction method was used to obtain the methanol extract of the leaf of *Combretum mucronatum.* The extract was analysed for antibacterial activities, using some pathogenic bacteria namely: *Klebsiella pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Bacillus cereus, Salmonella typhi* and *Bacillus subtilis*. The antibacterial bioassay was carried out *in-vitro* and it revealed that the methanol leaf extract inhibited the growth of the tested organisms at a concentration of 25.0 mg/ml except *Klebsiella pneumoniae* and *Streptococcus pyogenes* which were resistant. The extract exhibited the highest inhibitory potential on *Staphylococcus aureus* with a zone of inhibition value of 35.0 mm. This was followed by *Escherichia coli and Pseudomonas aeruginosa* which were inhibited with zones of inhibition values 30.0 mm and 25.0 mm respectively. *Bacillus cereus* was the least inhibited with a zone of inhibition of 16.0 mm. Result of the phytochemical screening tests revealed that the extract contains saponin, tannins, anthraquinone and cardiac glycoside. The rate at which the extract was able to kill the test organisms showed that the organisms decreased with increased time of exposure to the extract. *Pseudomonas aeruginosa* decreased to zero at the 24th hour. The minimum inhibitory concentration (MIC) of the leaf extract ranged from 25.0 mg/ml to 3.12 mg/ml. The result of the antibiotic sensitivity test compared well with the commercial antibiotics.

Key words; Antibacterial, Zone of inhibition, Phytochemical screening, Extracts, Rate of killing

Phytochemical and antibacterial properties of the leaf extracts of some edible plants in nigeria: Vernonia amygdalina, Ocimum gratissimum, Corchorous olitorious and Manihot palmate

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The antibacterial potency of ethanol, acetone and chloroform leaf extracts of Vernonia amygdalina, Ocimum gratissimum, Corchorous olitorius and Manihot palmata were investigated against ten bacterial isolates using the agar-well diffusion method. The bacterial isolates include Bacillus cereus, Escherichia coli, Salmonella Typhi, Pseudomonas aeruginosa ,Staphylococcus aureus, Shigella dysenteriae, Bacillus subtilis, Proteus vulgaris, Enterobacter aerogenes, and Clostridium sporogenes. The efficacy of the extracts against the bacteria was indicated by the appearance of clear zones of inhibition around the wells. The extracts except that of Corchorous olitirious showed inhibitory activities against Bacillus cereus, Salmonella typhi, Staphylococcus aureus, Shigella dysenteriae and Escherichia coli with zones of inhibition values ranging between 1mm and 20mm. The result of the activity of Vernonia amygdalina, Ocimum gratissimum and Manihot palmata against the organisms compared favourably with the activity of standard antibiotics. The rate of killing of the bacteria by the extracts over a period of eight hour interaction was also investigated. Results showed that the number of microbial cells were decreasing as the time of interaction between the extract and the bacteria increased. The Phytochemcial screening of the extracts revealed the presence of alkaloids, saponins, tannins, anthraquinones, steroids, flavonoids and cardiac glycosides.

Key words; Antimicrobial activities, extracts, zone of inhibiton, bacterial isolate, phytochemical components.

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Plant extracts application for the control of enteric *Salmonella* in egg farms

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Human illness caused by infection with enteric *Salmonella* dramatically increased worldwide since their first epidemiological tracking in the mid-1970s. *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) and serovar Enteritidis (*S.* Enteritidis) are the primary and predominant causes of foodborne salmonellosis, that routinely contaminate eggs, in the world. The hen house environment and the contaminated eggshells are from the main routes for *Salmonella* infection and transmission. The discovery and application of natural alternatives from plant origin to synthetic and chemical biocides is an increasing major global demand. The antimicrobial activity of eight plant extracts against *S.* Typhimurium and *S.* Enteritidis was in vitro determined using both quantitaive and qualitative assays. The most effective plant extracts, i.e. garlic (*Allium sativum*) leaves and pomegranate (*Punica granatum)* peels, were applied in laying farms as aerosol, for sanitizing flocks environment, and immersion solution, for disinfecting the experimentally contaminated eggs. The combination between the minimal inhibitory concentrations (MIC) from the two extracts increased their antibacterial potentiality and resulted in a complete inhibition of contaminating *S. enterica* growth. The application of plant extracts, however, could be recommended as eco-friendly, safe and powerful alternatives to the frequently applied chemical sanitizers and disinfectants against the invasion of enteric *Salmonella* in egg farms.

Keywords: antibacterial; garlic; pomegranate peel; laying farms; *S.* Enteritidis; *S.* Typhimurium

Polyphenolic compounds from *Larrea tridentata and Flourensia cernua* and their *in vitro* effect on *Rhizoctonia solani* mycelia inhibition

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The susceptibility of strains of *R. solani* resistant to the pencycuron fungicide was tested with plants extracts rich in polyphenolic compounds. These plant extract were obtained from plants (*Larrea tridentata and Flourensia cernua*) native of the Chihuahua Desert (Mexico) and using water, ethanol, lanolin and cocoa butter as solvents. Plant tissue was finely ground for the phytochemical extraction by infusion method used a ratio of tissue: volume (1:4) in a reflux system for 7 hours at 60 ° C. In the case of lanolin and cocoa butter were used emulsions. The concentration of polyphenolic compounds extracted (hydrolysable tannins and condensed tannins) was quantified by spectrophotometer at 725 and 460 nm. Antifungal activity of each extract was determined using poisoned culture medium technique at different concentrations (ppm) of polyphenolic compounds. In this case were placed mycelium disks of *R. solani* of 0.5 cm diameter with five days of active growth on PDA, and finally the Petri dishes were incubated at 25 ± 1 ° C and evaluated until the untreated control covered 100% of the Petri dish. The measured variable was the radial growth, which was transformed to percent mycelia inhibition. The experiment was established on a completely randomized design. Also Profit analysis was used to determine the plant extract concentrations inhibiting 50 and 90% of *Rhizoctonia solani* mycelia. The results showed that, the extraction of polyphenolic compounds is highly dependent on plant species and the solvent used. Also plant extracts exhibited an antifungal effect for up to 100% on *R. solani* mycelia growth inhibition. Polyphenolic compounds with the greatest impact are those obtained from *L. tridentate* using ethanol and lanolin and from *F. cernua* using ethanol and cocoa butter.

Keywords: polyphenolic compounds, plant extracts, antifungal properties, Rhizoctonia solani.

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Polyphenols from *Larrea tridentata, Fluorensia cernua*, *Agave lechuguilla* and *Lippia graveolens*: an alternative for control of *Clavibacter michiganensis* subsp. *michiganensis* and *Clavibacter michiganensis* subsp. *nebraskensis*.

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There were in vitro evaluated five different plant extracts against *C. m. subs michiganensis* (C. m. m.) and *C. m.* subsp. *nebraskensis* (C. m. n.). These extract were obtained from plants native of the Mexican Chihuahua desert using ethanol and lanolin as solvents. C. m. m. and C. m. n. strains were isolated from seed tomato and seed corn, respectively, purified and identified according to the criteria outlined by Shad et al. (2007) and identity was confirmed by ELISA. The *L. tridentate, F. cernua, A. lechuguilla* and *L. graveolens* extracts were obtained for the infusion method for 7h at 60 ° C using ethanol 70% as solvent and an emulsion in mineral oil (10%) was employed for lanolin in a ratio plant tissue: solvent (1:4). Concentration of total tannins (hydrolysable and condensed tannins) present in each extract was determined. The antibacterial activity of the extracts was determined using the poisoned culture medium technique to different concentrations of total tannins (50-2000 ppm), the King B medium was inoculated with 100 uL of a bacterial dilution to 1 x 10-4 of a colony of 12 hours of active growth on King B medium. The Petri dishes inoculated were incubated at 27 ° C for three days. The evaluation was performed at the time the colonies bacterial became visible in the treatment without extract (negative control) and counting the number of colonies on each repetition of the treatments. Data were transformed into percentage of inhibition of colonies. The data were analyzed under a completely randomized design with four replicates being used the Tukey test for mean comparisons.

The results varied for each bacterial species: for C. m. m. was found that the extracts at different concentrations inhibit the development of colonies in this sub-specie, a bactericidal effect was observed with the use of the lowest dose (50 ppm) of total tannins. According to the literature consulted, there were no references to the use of these plants extracts for control of this sub-specie, although it is reported that oils from marjoram (*Majorana hortensis*) leaves and flowers inhibited the growth of this subspecies in 80%, so it is reported an inhibition zone of 16 and 24 mm with 10 and 15 ug with essential oils of peppermint (*Mentha piperita*). For C. m. n. was observed that extracts of *L. tridentate* (ethanol and lanolin), *F. cernua* (ethanol) and *L. graveolens* (Lanolin) at different concentrations inhibited the development of colonies of this bacterium in 100%. However, for the extract *A. lechuguilla* (ethanol) at 50 and 150 ppm only had observed a bacterial static effect because it inhibits the development of the colonies until four days, after this time there is abundant growth of bacterial colonies inhibiting only about 1% to 50 ppm and 31 % at 150 ppm, the total inhibition was obtained at 300 ppm. We found no evidence of the use of plant extracts against this subspecies, so it is considered that this is the first report on the effect of these plant extracts on *C. m. nebraskensis.*

Keywords: plant extracts, bactericidal effect, Polyphenolic compounds, nonconventional organic solvents

Possibility of Using *Rosmarinus officinalis, Myrtus communis* and *Origanum sp*.'s essential oil as Fungicide in Pickling and Tanning Processes

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The objective of this study was to examine the applicability of *Rosmarinus officinalis, Myrtus communis* and *Origanum sp.* essential oil as fungicide against fungus that grows on leather during pickling and tanning processes. In the study, 2-n-Octyl-4-isothiazolin-3-one containing commercial fungicides were also used for control. During the microbiologic tests, the growth of mould species like *Aspergillus niger, Alternaria alternata, Penicillium chrysogenum* and *Trichoderma auroviride* that cause problems in leather industry were also investigated against these essential oil and fungicide.

As a result of this study, it has been determined that oregano essential oil has most effective antifungal activity.

Keywords Leather industry, Pickling, Tanning, Essential oil, *Rosmarinus officinalis, Myrtus communis* and *Origanum sp.*

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Protection of organic animal feedings from mycotoxigenic aspergilli invasion via the application of antifungal plant extracts

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Organic farming requires minimal or none chemical pesticides application. Moist animal feed are normally susceptible to the attack by mycotoxigenic and spoilage fungi during their preparation and storage, this invasion become more vigorous within feeds prepared from organic-farmed plants. As the global trend is rapidly shifting toward the substitution of synthetic biocides with natural, biodegradable and eco-friendly alternatives, so that the determination of antifungal activity of many plants extract against mycotoxigenic *Aspergillus flavus* and *A. ochraceous* was carried out in current study. Many examined plants exhibited potent antifungal activity using in vitro assays. The GC-MS analysis of the plants extract clarified their main active constituents. The most effectual biocidal plant extracts, i.e. garden cress seeds (*Lepidium sativum*), garlic leaves (*Allium sativum*) and khella seeds (*Ammi visnaga)*, were applied with their minimal inhibitory concentrations (MIC) during feed preparation. The growth of inoculated fungi was completely inhibited with synergistic application of the three extracts MICs during feed storage for 21 days. The biosafety of formulated feed was confirmed by feeding experimental animals, i.e. mice and sheep, with treated feeds for 30 days. The application of plant extracts, however, could be recommended in the preparation of moist organic animal feeds as safe and natural alternatives to synthetic fungicidal agents.

Keywords antifungal; Aspergillus; biosafety; natural biocides; organic feeding

Screening of Aqueous Methanol Plant Extracts for Their Antibacterial Activity

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The purpose of this experimental study was to observe the antibacterial effect of aqueous methanol extracts of ten local Pakistani plants against two gram negative bacteria, *(Escherichia coli, Pasturella multocida*) and three gram positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Corynebacterium bovis)* by using disc diffusion method. The minimum inhibitory concentration (MIC) was determined by agar well diffusion method and agar dilution method. All the bacteria were susceptible to different plant extracts. *Impactien balsamina* L, *Embellia ribes* Burn and *Santalum album L showed* antibacterial activity against all the tested bacteria. The extract of *Santalum album* was most effective antibacterial activity of the ten plant extracts used. *Bacillus cereu* and *Pasteurella multocida* were the most sensitive bacteria against most of the plant extracts.While *Mallotus phillipensis, Colotropis procera*, *Carum copticum, Ricinus cummanis, Amomum subulatum, Operculina turpethum, Citrullus colocynthis* different effect on different bacteria. The present study will be helpful for the local community, traditional healers and those who involved in the study of ethnomedicine.

Keywords; minimum inhibitory concentration, agar well diffusion, agar dilution method

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Selective growth inhibitory effect of plant derived compounds against intestinal tract colonizing bacteria

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Clostridium species are frequently related to intestinal disorders occurring in humans as well as in animals and to food poisoning and spoilage [1, 2]. The enormous medical and economic impact associated with clostridia have assumed worrying proportions [3]. Since the plants are known to produce antimicrobially effective constituents they have been extensively studied as promising sources of human disease-controlling agents [4] without a harmful effect to the positive gut microbiota.

In this study we evaluated 21 plant-derived substances for their selective growth-inhibitory activity against clostridial pathogens of intestinal tract (selected type cultures*: Clostridium butyricum, Cl. clostridioforme, Cl. paraputrificum, Cl. perfringens,* and *Cl. tertium*) and simultaneously towards beneficial intestinal microflora represented by bifidobacteria (selected type cultures: *Bifidobacterium animalis*, *Bif. bifidum, Bif. catenulatum, Bif. longum*) using the broth microdilution method [5] and expressed as minimum inhibitory concentration (MIC). Tetracycline was assayed as control antibiotic.

From the 21 substances, four possessed selective antimicrobial effect, namely curcumin (CC), nordihydroguaiaretic acid (NDGA), thymoquinone (TQ) and withaferin A (WA). The results showed that none of these substances restrained bifidobacteria even at the highest concentration tested (MIC $\geq$ 1024 μM), while particular compounds significantly inhibited certain clostridia where MICs were determined as follows: TQ $\geq$ 64 μM, CC $\geq$ 256 μM, NDGA = 512 μM, and WA = 512 μM.

Our results suggest the potent selective antimicrobial properties of TQ, CC, NDGA and WA against clostridia whilst not affecting bifidobacteria.

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Keywords: selective antimicrobial activity, intestinal infection, clostridia, bifidobacteria

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Sporothrix schenckii: effect of selective inhibitors of glucosamine-6-phosphate synthase on activity of purified enzyme and on fungal growth

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All four enzymes leading from fructose-6-phosphate to UDP-GlcNAc in the Leloir pathway are considered as potential targets for antifungal chemotherapy. However, only glucosamine-6-phosphate synthase (L-glutamine:D-fructose-6-phosphate amidotransferase; EC 2.6.1.16; GlcN-6-P synthase) has been extensively studied in this context. This ubiquitous enzyme catalyzes the first committed step of hexosamine biosynthesis, converting fructose-6-phosphate into glucosamine-6-phosphate using L-glutamine as the ammonia donor. A distinctive character of GlcN-6-P synthase is its extreme instability which has seriously limited purification of the native enzyme to homogeneity. In fungi, overexpression of the respective genes in different hosts has allowed its purification and characterization in *Saccharomyces cerevisiae* and *Candida albicans*. The enzyme from *C. albicans* is by far one of the best studied at the structural, functional and regulatory levels. We succeeded in the purification and partial characterization of the native enzyme from *S. schenckii*, a truly dimorphic fungus and the etiological agent of human sporotrichosis. This mycosis, whose incidence has increased over the last few years particularly in immunocompromised patients, is acquired by traumatic implantation of the organism and it is characterized by nodular lesions of cutaneous and subcutaneous tissues with lymphatic involvement. More recently, we also purified the recombinant His_6tagged-enzyme overexpressed in *S. cerevisiae* by metal affinity chromatography and compared some of its properties with the native counterpart.

Development of more specific, less toxic and potent inhibitors of GlcN-6-P synthase has allowed the synthesis of a number of antifungal compounds. Two of these are FMDP [N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid] and ADMP (2-amino-2-deoxy-D-mannitol-6-phosphate) which are analogs of L-glutamine and the putative transition state intermediate, respectively. In contrast with previous reports indicating that ADMP (and also ADGP) are the strongest inhibitors of the fungal enzyme, we observed that FMDP was a stronger inhibitor of purified GlcN-6-P synthase from *S. schenckii* than ADMP. However, ADMP and two lipophylic derivatives of ADGP (N-butanoil- and N-hexanoil-ADGP) were more potent inhibitors of fungal growth than FMDP and two FMDP-derived oligopeptides (Nva-FMDP and Nva-Lys-FMDP) in YNB and RPMI culture media. These results are discussed in terms of possible differences between *S. schenckii* and other fungi.

Keywords *Sporothrix schenckii*; GlcN-6-P synthase; antifungal target, enzyme inhibitors

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Tea Tree Oil, Plant Essential Oil components and their mixtures for the control of phytopathogen and mycotoxigenic fungi in crops.

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It is worldwide recognized that the full yield potential of crops cannot be expressed, because limited by abiotic and biotic stresses. In particular, plant pathogen fungi can cause relevant losses in terms of yield, quality and safety. Moreover, some phytopagenic fungi produce different classes of mycotoxins, that can persist along the whole agri-food chains, with a strongly negative impact on human and animal health. Starting from these considerations, it is clear that research activities focused on the development of sustainable strategies for crop protection are needed because emerging, re-emerging and endemic plant pathogens continue to challenge our ability to safeguard plant health worldwide. Because the future of crop protection should lie in a combination of several different tools and strategies, alternative measures to synthetic pesticides have been developed, including biological agents, mineral salts and plant extracts. Among these, the complex mixtures of compounds, mainly monoterpenes and sesquiterpenes, that characterized the chemical composition of essential oils can potentially be considered as alternative natural fungicides.

In this study, the activity of *Melaleuca* essential oil (Tea Tree Oil, TTO), of some major essential oil components and of different mixtures TTO plus oil component/s has been evaluated for the control of different classes of plant pathogen fungi that affect small grain cereals and other crops widely cultivated in Mediterranean environments. The fungi considered are characterized by different life cycle, pathogenicity behavior, transmission mode and effects on plants and derived agro-food products. The antifungal activities of TTO, of single oil components and of mixtures have been evaluated *in vitro*, determining the impact of different concentrations of the substances on the fungal growth. Different potency in fungal growth inhibition of the different oil components have been found. The effects of TTO, single oil components and mixtures have been studied *in vivo*, on cereal leaves maintained in controlled conditions of environment and infection. In these conditions, the possible role of TTO as elicitor has been evaluated with functional genomic approaches.Finally, the protective activities of TTO, single oil components and mixtures have been evaluated in open field trials using susceptible cereal varieties.

Keywords crop protection; essential oils

The Antibacterial Activity of *Phaeobacter inhibens* KJ-2 isolated from the marine organism Sea hare eggs.

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This study was conducted to investigate the possible use of probiotics in fish farming by evaluating the in vitro antagonism of antimicrobialstrain *Phaeobacter inhibens* KJ-2 strain against the fish-pathogenic bacterium *Vibrio anguillarum*. A bacterial strain that showed ability to produce antimicrobial compound was isolated from marine organism Sea hare eggs.

Identified bacterial strain was named as *Phaeobacter inhibens* KJ-2basedon The biochemical characterization and16S rRNA sequence analysis. Phenotypic classification results showed that *Phaeobacter inhibens* KJ-2 could be classified as a aerobic, Gram-negative, motile bacteria that forms brown-pigmented colonies. Production of antimicrobial compound and higher

Growth of *P. inhibens* KJ-2were observed at 20℃ for 24 hours.

Optimum conditions for production antimicrobial compound of *P.inhibens* KJ-2 were determined as media containing 1.5% sorbitol, 0.8% NH_4NO_3 4% NaCl with pH 6.0 and temperature at 20℃. However, production of Antimicrobial compound was inhibited by mineral sources. A antimicrobial compound of *P. inhibens* KJ-2 was stable within the pH range from 3～10 and temperature ranging from 40～121℃. However, antimicrobial activity was decreased pH range from 9～10. Therefore, our results confirmed that antibiotic compound isolated from P.inhibensKJ-2 has provided a potential biological agent for controlling fish pathogenic bacteria.

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The Effectiveness of Betel, Thyme and Garlic as Antifungal Agents

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Introduction: Thyme and garlic are widely consumed as condiments in the preparation of foods. The extracts of thyme and garlic produce very strong flavour due to the presence of compounds such as thymol and allicin, respectively. Together with betel, thyme and garlic have also been reported to exhibit some growth inhibition activities towards yeasts. Thyme is effective against fungi when applied topically on fungal-infected toenails. Garlic and betel have been reported to possess a wide spectrum of activities including anti-bacterial, anti-viral and anti-fungal. *Candida* is a genus of yeasts with its members often referred to as the imperfect fungi. In humans with healthy condition, *Candida* forms a very small percentage of the normal flora of skin, mouth and vagina. In a disturb ecosystem however, an over growth of *Candida* often leads to opportunistic mycoses in the mouth and vagina. The aim of the study was to investigate the effectiveness of the extracts of betel, thyme and garlic in killing or inhibiting the growth of *C. albicans, C. tropicalis* and *C. krusei. Methodology:* 25 g of fresh leaves of betel and thyme, and bulbs of garlic were cleaned, crushed and then immersed in 100 ml of ethanol in screw-capped dark bottles. After two weeks, the solvent containing the extracts, which were then referred to as tinctures, were used in anti-fungal assays against *C. albicans, C. tropicalis* and *C. krusei*. The broth dilution method was performed to determine the minimal inhibition concentrations (MIC) of the tinctures. A time-kill study was carried out to determine the effectiveness of the tinctures in producing growth-inhibiting effect on the candidal cells. For the assay, cell suspensions of *C. albicans, C. tropicalis* and *C. krusei* were prepared by inoculating three growth colonies of each species from an agar plate into 10 ml of sterile saline. Following a 60 s sonication to break the colonies and disperse the cells, 0.5 ml of the suspension was pipetted out and introduced into 5 ml of thioglycollate broth which have been added with 0.5 ml of the respective tinctures. The culture tubes were then incubated in a water bath set at 37 °C. At the 0, 1, 2, 3, 8, 21 and 24 hrs of incubation, 100 µl aliquots of the growth suspension was taken and spread on PDA plates. The colony forming units following 24 hr incubation at 37 °C was enumerated. *Results and Discussion:* Tinctures prepared from betel and thyme showed equivalent growth inhibitory activity towards *C. albicans, C. tropicalis* and *C. krusei* with MICs at 3.91 mg ml^{-1}. The tincture prepared from garlic exhibited the lowest MIC at 0.98 mg ml^{-1} towards all three *Candida* spp. with growth inhibitory activity 4 times higher than that of betel and thyme. Based on the time-kill test, minor inhibitory actions were observed by all the tinctures on *C. albicans, C. tropicalis* and *C. krusei* for the first 3 hrs following exposure of the cell suspensions to the tinctures. In general more than 50 % cell growth was inhibited within 8 hrs following the treatment. Garlic tincture was most effective in killing *C. albicans* (94.5%) followed by *C. tropicalis* (91.9%). Betel tincture was more effective towards *C. albicans* (76.8%) than to *C. tropicalis* (52.5%) and *C. krusei* (42.1%). Thyme displayed a low inhibitory activity towards all three *Candida* spp. Based on these *in vitro* observations, garlic and betel preparations have potential to be incorporated as active ingredients in cream of gel which can be applied topically to treat skin with fungal infection. Both would also be suitable ingredients in mouth or vaginal washes to control the over growth of *Candida* that causes mycoses. *Conclusion:* Betel, thyme and garlic exhibited varying effectiveness towards *C. albicans, C. tropicalis* and *C. krusei* as anti-fungal agents. The anti-fungal activity of garlic and betel was more effective towards *C. albicans* while thyme was more towards *C. tropicalis*. *C. krusei* showed low susceptibility towards all three plants.

Keywords *C. tropicalis; C. krusei; C. albicans;* time-kill assay; growth inhibition

The investigation of antimicrobial activities of Rosmrinus officinalis L. essential oil in lemon beverage

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Increasing awareness of consumers during last decades resulted in using safe additives specially anti-microbial components in food products. Rosemary herb is a rich source of natural antioxidant and contains phenolic and terpen compounds with anti-microbial capacity. In this research a lemon flavor beverage preserved with natural rosemary essential oil was produced and some parameters such as: minimum inhibitory concentration (MIC), growth rate of aerobic mesophilic bacteria, yeast and acid-resistant micro-organisms have been evaluated. Concentration of 1000, 1500 and 2000 ppm of rosemary and a sample containing 2000 ppm of rosemary + 50 % benzoate was used to control growth of spoiling bacteria (*L. mesenteroides, L. Delbrukii*) and yeasts (*Sac. Cervisiea, Candida cruseii*) in the beverage. Samples enriched with rosemary essential oil and blank sample were kept at 4º C and room temperature. Results showed that the growth of spoiling microorganisms weakened in room temperature compared with those kept at 4º C. Microbial tests showed that the use of rosemary essential oil preserves the lemon beverage during 90 days without any negative effects on the organoleptic properties of the beverage.

Keywords: Rosemary; lemon beverage; antimicrobial activities

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Vapours of monoterpenes at sublethal doses elevate virulence of the plant pathogenic bacterium *Pectobacterium carotovorum*

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The antimicrobial properties of essential oils (EOs) have long been suggested as agents in human and animal therapy. One of the advantages of EOs is their bioactivity in the vapour phase, making them useful as possible fumigants for stored commodity protection and modified atmosphere techniques. Here we investigate whether exposure of the plant pathogenic bacterium *Pectobacterium carotovorum* to sublethal doses of monoterpenes (the most investigated EOs components) can lead to virulence modulation. Cabbage leaf discs or celery petioles were pierced at the centre with a sterile tip, inoculated with 10 µl of mid-log-phase of selected bacterial cultures (OD = 0.5, ~10^8 cfu/ml) and placed in Petri dishes containing 15 ml of 1/2 Murashige and Skoog mineral sugar-free agar medium. A filter paper soaked with a selected monoterpene was taped to the lid of the Petri dish. The dish was then sealed with parafilm™ and incubated at 24°C for 24 h, after which the necrotic area was measured.

Exposing *P. carotovorum* cells to sublethal levels of menthol, limonene or thymol in the vapour phase enhanced their virulence in both plant models. In cabbage, the presence of menthol vapour increased the necrotic area 5.6 fold (92.7 mm^2 in the presence of menthol as compared to 16.4 mm^2 for the control). An increase of 4.2 fold in necrotic area was obtained also under the same conditions in celery petioles. A Similar pattern was observed when the two plant models were exposed to sublethal levels of either thymol or limonene. Exposure of *P. carotovorum* cells plated on pectin supplemented growth medium and exposed to sublethal doses of menthol revealed a significant increase in pectin methyl esterase and polygalacturonase activity as compared to the non-exposed control. Current work is aimed at identifying the molecular mechanism modulating the "smelling" of the essential oil and the increase in the bacterium virulence.

Keywords: *Pectobacterium* carotovorum, menthol, limonene, thymol, virulence

- Menthol

+ Menthol

5. Antimicrobial surfaces - Biofilms - Quorum sensing - Consumer products

A novel dental adhesive with bioactive and on-demand biofilm eliminating properties

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Biofilms are a prevalent mode of microbial life found in nature. Bacteria in biofilms are 10-1000 times more resistant to antibiotics than when in planktonic form, and in many cases are developing resistances to existing antibiotics; as such, there is a growing requirement for new strategies in biofilm elimination. Dental plaque is an example of a biofilm that often results in dental diseases such as caries. Furthermore, dental plaque is often associated with restorative dentistry materials, which often enhance and increase the accumulation of bacteria. For example, cracks that develop between the filler and the tooth offer ideal conditions for biofilm development that leads to secondary caries. The aim of the present work was to perform an in vitro evaluation of a novel dental adhesive containing photocatalytic TiO_2 nanoparticles for bioactivity and on-demand biofilm elimination through ultraviolet (UV-A) irradiation.

The dental adhesive was prepared by adding 20 wt% TiO_2 nanoparticles to a light cured resin matrix of HEMA and bis-GMA polymers. Spontaneous hydroxylapatite formation on the surface of the adhesive samples upon storage in simulated body fluid indicated good bioactive properties, and suggests that the material should better integrate with the adjacent tooth tissue. Biofilm elimination testing of the adhesives was accomplished by irradiating the biofilm-coated surface of the photocatalytic adhesive with UV-A light. Results showed that a dose of approximately 6 J/cm^2 led to a 1 log reduction in the concentration of viable bacteria in a biofilm that was grown on the surface of the adhesives. As much as 7 log reduction in bacteria was achieved with a total UV-A dose of 45 J/cm^2.

The combined features of bioactivity and on-demand bactericidal effect open the potential to create dental adhesives that reduce the incidence of secondary caries by promoting closure of gaps forming at the interface towards the tooth via remineralization of adjacent tooth substance, as well as elimination of bacteria growing in dental plaque adherent to such dental materials.

Keywords dental adhesive; biofilm; titanium dioxide; photocatalysis; bioactivity; antibacterial

ADJUSTING THE ANTIMICROBIAL PROPERTIES OF SILVER NANOPARTICLES WITHIN A FUNCTIONAL PLASMA POLYMER MATRIX

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Silver (Ag) is a widely used antibacterial agent in medical devices or wound dressings. However, cytotoxicity and hindered healing effects are also associated with Ag products. Therefore, new Ag containing products with an application based optimised antibacterial yet still cytocompatible Ag release are needed to overcome these problems.

Plasma technology enables the production of Ag nanocomposites with precise adjustable properties. Functional hydrocarbon plasma polymer coatings with embedded Ag particles were deposited using an asymmetric RF plasma reactor at low pressure (10 Pa). The plasma polymer is produced with a reactive gas/monomer mixture of CO_2/C_2H_4. Ar was added in order to sputter Ag atoms from the Ag cathode and form nanoparticles in the growing polymer matrix. The properties of the Ag nanocomposites can be adjusted due to adapted process parameters within this one-step plasma process. One focus is besides the plasma polymer nanostructuring the Ag content and Ag distribution in the polymer matrix. The Ag content can be adjusted due to the gas ratio C_2H_4 and CO_2 and the power input (Fig. 1). An increasing Ag content of the coatings consequentially yields a higher Ag release over a timescale of 14 days. A Ag gradient in the nanocomposites leads to more continuous Ag release.

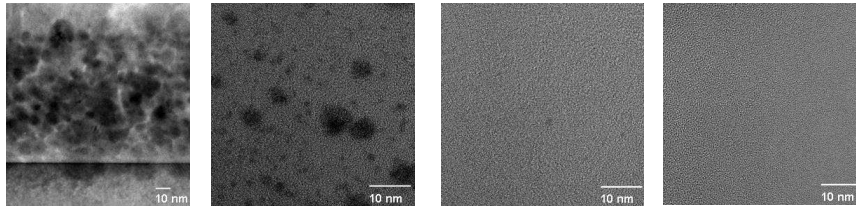

Fig. 1: Ag nanoparticle distribution for various plasma process parameter a) CO_2/C_2H_4 6:1 - 100 W, b) CO_2/C_2H_4 6:1 - 50 W, c) CO_2/C_2H_4 2:1 - 50 W and d) CO_2/C_2H_4 2:1 - 30 W

The coatings exhibit an excellent effectiveness against the *P. aeruginosa.* Although these coatings show the lowest Ag concentration and thus smallest Ag release within the examined range, no bacterial surface contamination could be observed. A higher amount of Ag, on the other hand, was found to be required for *S aureus*. The coatings with a Ag content lower than 0.1 g/cm^3 or with a Ag gradient were found to be cytocompatible (Fig. 2).

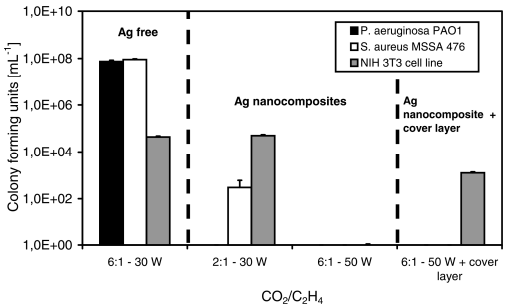

Fig. 2: Bacteria and cell growth on plasma polymer coatings and Ag nanocomposites

Keywords silver, nanocomposite, release, cytocompatible

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An Analysis of the Proteomic Response of *Staphylococcus aureus* to Silver(I)

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Staphylococcus aureus is a Gram positive, versatile pathogen which has the ability to survive in nutrient-limiting and stressful conditions. Hospital acquired infections by *S. aureus* can take place by direct contact with a wound, airborne carriage and contact with indwelling devices, such as catheters. Approximately 30% of healthy individuals carry *S. aureus*, most commonly in the anterior nares (i.e. nose), but it can lead to a wide range of skin and wound infections and also to more serious diseases, such as toxic shock syndrome (TSS) and sepsis. Due to the increasing resistance of bacteria to antibiotics there has been a renewed interest in using metal coordination complexes as antimicrobial agents, in particular silver(I) complexes**.** Silver(I) ions are now used in many topical wound creams (e.g. silver sulfadiazine), in bandages for wounds and they are also incorporated into indwelling catheters and medical devices.

The aim of the present work was to examine the interaction of silver(I) ions with *S. aureus* which is the main colonizer of wounds. We have shown that exposure of the organism to silver(I) ions leads to an increase in the leakage of amino acids from the bacterial cells and creates a bacteristatic effect. It was also found that there was an increase in the activity of superoxide dismutase and glutathione reductase in response to silver(I). 2-D SDS-PAGE electrophoresis was performed to identify some of the proteins that were released from the silver(I)-treated cells and also those proteins that were altered in expression following exposure to the metal ion. It was found that the expression of certain external proteins, such as alpha-hemolysin, which is the main pore forming toxin of *S.aureus*, was increased 2-fold. In addition, a putative universal stress protein, which indicated that the cells are under stress as a result of exposure to the silver(I) ions, also increased 2-fold. It was also observed that there was a smaller increase (0.8 fold) in the expression of internal proteins, such as SOD A, which correlates with the results of earlier enzymatic studies. Silver(I) ions have potent anti-bacterial activity and this work demonstrates that cells attempt to mount a protective response against them. Our results indicated that there was an increase in a number of proteins associated with the cell's response to oxidative and osmotic stress, suggesting the possibility of a resistance or 'protective' response of the cells to the metal ion.

Keywords *Staphylococcus aureus*; Silver(I); protective Response

Anti-Quorum Sensing and Anti-Oxidant Activities of *Funtumia elastica* (Preuss) Stapf (Apocynaceae).

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Investigation into the anti-quorum sensing and anti-oxidant activities of methanolic extracts of the leaf and the bark of *Funtumia elastica* has been conducted. *F. elastica,* also known as the West African Rubber tree, is a tropical plant belonging to the family Apocynaceae and is found in the western coast of Africa. In Ghana and other parts of Africa it is used in treating sexually transmitted diseases (STDs), dysentery, whooping cough as well as management of wound.

Methanolic extracts of *F. elastica* leaf and bark at 5mg/ml, exhibited anti-quorum sensing activity of *Chromobacterium violaceum* by inhibiting the production of purple coloured violacein in response to quorum sensing without affecting the growth of the organisms cultured on Muller-Hinton medium. In addition, the petroleum ether, ethyl acetate, butan-1-ol and aqueous fractions partitioned from the methanolic extract of the leaf, at the same concentration of 5mg/ml, also exhibited loss of purple colouration. The butan-1-ol and aqueous fractions exhibited the greatest ability of purple colour (violacein) inhibition, and greatest anti-QS activity. Furthermore, all the extracts and the fractions exhibited a potent anti-oxidant activity using the DPPH assay system; their activities being greater than that of α-tocopherol, a standard anti-oxidant compound.

The ability of the plant and its fractions to interfere with the communication network of the organisms (quorum sensing) suggests that this activity may contribute to the purported antimicrobial activity of *F. elastica* in folklore medicine. This stems from the fact that virulence factors of most pathogenic bacteria are under the control of quorum sensing.

The study has shown that in the search for compounds capable of attenuating microbial pathogenicity, through mechanisms such as interference with the cell-to-cell signalling molecules, terrestrial plants with antimicrobial activities could be a potential source.

Keywords: Antimicrobial; Antioxidant; Apocynaceae; *Chromobacterium violacium; Funtumia elastica*; Quorum sensing; α-tocopherol; violacein.

Antibacterial activity of bone cement containing quaternary ammonium polyethylenimine nanoparticles.

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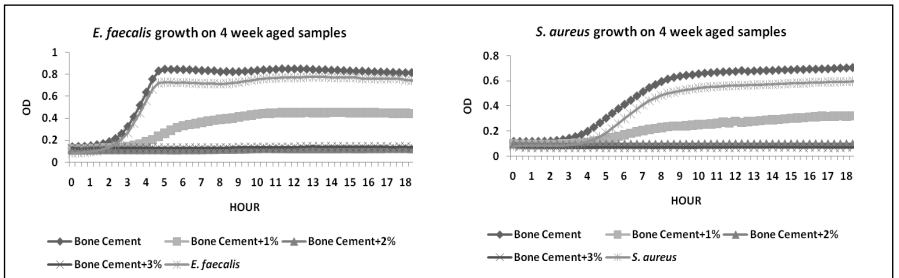
Bacterial infection following joint replacement surgery is a catastrophic complication. It is estimated that infection occurs in 1-2% of primary hip and knee replacements, and twice as aften in revision surgeries. These infections carry significant morbidity and mortality as well as a huge economic burden estimated at hundreds of millions of USD annually in the US alone. Early infections are considered to be related to intraoperative innoculation. Antibiotic-loaded bone cement is commonly used among other preventive measures, however the antimicrobial effect is limitted to the first two weeks in which antibiotic is released from the cement. Late infections are more common. Sometimes they may be attributed to hematogenous spread of other acute infections but most commonly the exact rout and time of infections are unknown. Both skin flora and enteric bacteriae are known to proliferate in the bone-cement-implant interface, where native immune response and antibiotic perfusion are diminished.

The purpose of this study was to modify the commonly used bone-cement to gain safe and lasting antibacterial effect. We hypothesized that mixing quaternary ammonium polyethylenimine (QPEI) nanoparticles into bone cement will result in a lasting, dose-dependent increae in antibacterial activity. To this end, we tested the antimicrobial effect of insoluble crosslinked quaternary ammonium polyethylenimine (QPEI) nanoparticles incorporated at 1, 2 or 3% w/w in a bone cement (PMMA - polymethylmethacrylate). The antibacterial effect against *Staphylococcus aureus* and *Enterococcus faecalis* was tested using the direct contact test (DCT) and agar diffusion test (ADT).

Using the direct contact test, antibacterial activity was significant in all three formulations of PMMA with QPEI nanoparticles ($p < 0.05$). The antimicrobial effect was still significant when samples were aged for 4 weeks. ADT showed no inhibition halo in the plate for the three formulations for both test bacteria, indicating that potentially toxic antimicrobial nanoparticles are retained in the PMMA and do not diffuse into the agar milieu.



Bone cement incorporating QPEI inhibits growth of *E. Faecalis* and *S. Aureus*: Kinetic measurements of bacterial growth following direct contact between *E. faecalis* (left) and S. Aureus (right) with 4 week-aged bone cement with 0,1,2 and 3 % w/w incorporated QPEI nanoparticles.Growth of the shed bacteria from the biofilm was measured every 20 min for 18 hrs. Each point on the curve is the average (±SD) optical density absorbance (OD) measured in 8 replica wells similarly prepared in the same microtiter plate.

Bone cement is commonly used in orthopedic surgery and specifically in arthroplasty to acheive fixation of innert prosthesis to host bone. The cement mantle surounds the prosthesis and paradoxically isolates the foriegn body from the protective effect of the host immune system, thus creating an ideal environment for bacterial growth and biofilm formation. Thus, bone cement possessing antibacterial properties may be beneficial. Our results indicate that incorporation of QPEI nanoparticles in bone cements has a long lasing antibacterial effect that, in turn, may prevent early and late prosthetic joint infections and prolong their clinical performance.

Keywords antibacterial, bone-cement, quaternary ammonium polyethylenimine.

Antibacterial activity of dental glass ionomer cements incorporating quaternary ammonium polyethylenimine nanoparticles against *Streptococcus mutans* and *Lactobacillus casei*

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Although glass ionomer cements (GIC) serve as sealing agents at the margins of fixed partial dentures (FPD) possible microleakage may occur. Gap formation and microleakage are not completely prevented even when using state-of-the-art bonding systems. Gaps between restoration margins and cavity walls are colonized by oral microorganisms and may result in secondary caries and pulp disease. Secondary caries has been identified as the major factor that influences the longevity of dental restorations. Thus, growth-inhibitory effect of cements and bonding systems are considered to be beneficial in preventing bacterial colonization of marginal gaps. Previously, we showed that incorporation of small amounts of quaternary ammonium polyethylenimine (QPEI) antibacterial nanoparticles into resin composites render a strong antibacterial effect against a wide range of bacteria for at least a one month with no measured effect on biocompatibility. Furthermore, we reported that small amounts of QPEI nanoparticles can be immobilized into resin composites during polymerization without leaching-out and without compromising the mechanical properties of the resin composite. In the present study we further investigated QPEI nanoparticles when incorporated in two conventional GICs. The objective of this study was to investigate the antibacterial effect of GICs incorporating 1% w/w QPEI on two cariogenic bacteria: *Streptococcus mutans* and *Lactobacillus casei*. The antibacterial activity was tested against *Streptococcus mutans* and *Lactobacillus casei* using the direct contact test (DCT) and the agar diffusion test (ADT). Using the direct contact test, antibacterial activity ($p < 0.05$) was found in both GIC's tested incorporating QPEI nanoparticles (see fig.) The effect lasted for at least one month.

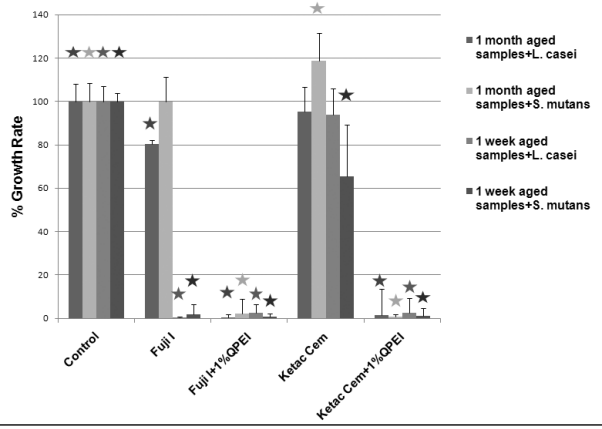

Fig. Bacterial growth rate (in percentage) of *Streptococcus mutans* and *Lactobacillus casei* following direct contact with aged glass ionomer cements (Fuji I and Ketac Cem) with 0 and 1% w/w incorporated QPEI nanoparticles. Growth rate determined in separate experiments in which samples were aged for 1 week or 1 month. The results are expressed as the mean ±SD, significant differences between the appropriate control and the different test groups are indicated by an asterisk ($p < 0.05$).

However, ADT showed no inhibition halo in both test bacteria, indicating the antimicrobial nanoparticles are not diffusing into the agar. The results indicate that incorporation of QPEI nanoparticles in glass ionomer cements has a long lasing antibacterial effect against *Streptococcus mutans* and *Lactobacillus casei*. The addition of antibacterial quaternary ammonium polyethylenimine nanoparticles into glass ionomer cements may be an excellent tool to prolong the clinical performance of FPDs.

Keywords polyethylenimine; nanoparticles; antibacterial; glass ionomer cements

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Antibacterial activity of Photocatalyst coating on ceramic foams for the application to air cleaner

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Background: During the past decades, the air-borne diseases, such as the "SARS" outbreaked in 2003 in China and the international epidemic "Influenza" in 2009, seriously threatened human health. Recently, the application of Titanium dioxide (TiO_2) photocatalyst in air purification systems has attracted much attention as a measure for this problem because of its strong bactericidal activity. This activity is relative to the strong oxidizing power of TiO_2 with the combined action of hydroxyl radical ($OH^{\bullet}$) "produced by oxidation of water" and superoxide radicals ($O_2^{\bullet-}$) "produced by reduction of oxygen under UV light illumination on it. In the present study, we developed a novel kind of air-cleaner filter with a highly photocatalytic performance by coating TiO_2 on ceramic foam, and examined its bactericidal properties against *E. coli*, *Legionella pneumophila*, *Klebsiella pneumoniae* and MRSA.

Metials and Methods: The TiO_2 was coated on the surface of the ceramic foam substrae by a sol-gel process. The foams made from cordierite material ($2MgO/2Al_2O_3/5SiO_3$) with 80 ~ 88% porosity were immersed in a TiO_2 slurry. After removing the residul sol on the socked foams, they were calcined at a high temperature to satisfy the two conflicting criteria: higher mechanical strenghth and larger surface aerea of the coating film.

The phtotcatalytic inactivation on bacteria of the TiO_2-coated ceramic foams were investigated by an original test method designed with a simulation of the actual use conditions of TiO_2/ceramic foams.The created foams were soaked in the bacteria suspentions to absorb the testing bacteria cells on its surface at a optimum conditions for adsorption equilibrium. Then, they were treated to a semi-dry status by a centrifugal action, and were irradiated with a UVA light at a low intensity ($0.25mW/cm^2$) in the room temperature. After a period of time for irradiation, the bacteria cells absorbed were collected with washing the foams by PBS-Tween20 solutions. The viable bacteria cells in the washing solutions were analyzed by colony formation unit (CFU) detection to evaluate the bactericidal rate of photocatalytic ceramic foams.

Results and dicussion: By creating optimum conditions including higher temprature calcination, TiO_2 nano-particles were immobilized firmly on the surface of ceramic foams to prevent scattering of the nano-particles. Simultaneously, the created foams exhibit a high bactericidal efficiency on the various bacterial strains described above. Here, we display a representative data as shown in the figure: by $0.25mW/cm^2$ intensity of UVA irradiation for 24h, the bactericidal rate of the TiO_2/ceramic foams on *L. pneumophila* was achieved to 99.9%. Aditionally, the results of repeated tests for the same sample indicated that the created foam possesses a long-term bactericidal effect and a reusability. The bactericidal tests were repeated a minimum of three times for each strain by our developed assessment method, and all data showed a very higher reproducibility.

All of these results explain that the TiO_2-coated ceramic foam can be used to remove airborne bacteria.

Keywords: Photocatalytic air-cleaner; Ceramic foam; Bactericidal activity; Air-borne diseases prevention

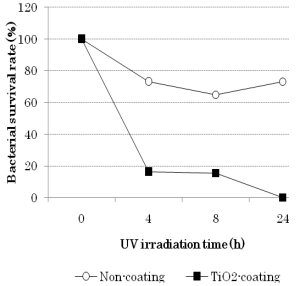

Fig. Bacterial effect of the TO_2-coated ceramic foam against *Legionel la pneumophila* with UVA irradiation.

Antibacterial actvity of nano silver in the floricultural industry

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Floriculture is one of the most important branches in agriculture. Many countries produce several types of cut-flowers such as rose, chrysanthemum, carnation, lilium and gerbera in the floriculture. After harvesting of these cut-flowers, they are alive and need preservative solutions which containing water and food resources (i.e. sucrose). On the other hand, food resources promote microbial growth in preservative solutions. Commonly, chemical components such as silver nitrate are used for inhibition of microbial infection. In this research, we studied the effects of four concentrations of nano silver as new antimicrobial component (0, 2, 5 and 7 ppm), two concentrations of sucrose as food resources (4 and 6 %) and four sampling date (1, 4, 7 and 11) in factorial concept based on completely randomized design on bacterial colony formation from preservative solutions. The results showed that, application of nano silver, particularly in high concentrations, inhibited bacterial growth. Whereas treatments which containing both concentrations of sucrose (without nano silver) induce bacterial growth. We concluded that, nano silver as new antibacterial have a good potential for inhibiting bacterial growth in the preservative solutions in the floriculture industry.

Keywords: microbial infection, nano material, bacterial population, floriculture

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Antibacterial mechanisms of nanohybrid of the immobilized silver nanoparticles and exfoliated platelet clay

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Nanohybrid consisted of silver nanoparticles and exfoliated platelet clay from Na^+-montmorillonite, exhibit high potential to inhibit bacterial growth. Silver nanoparticles (AgNP/NSP) has great efficacy on antimicrobial activity but tend to self-aggregation status in aqueous environment. Thus a nanosilicate platelet was used as a capping agent in the process of synthesis silver nanoparticles preventing it from self-aggregation and in situ immobilized the particles. The plate-like clay, due to its charged and large surface area can physical attach to bacterial membrane. With silver nanoparticles which immobilized on surface, this novel nanohybrid killing bacterial cells efficiently, including Ag^+-resistant bacteria J53pMG101. Silver resistance plasmid pMG101 provide bacterial cells ability to pump out silver ions that entering the cells through two parallel efflux pumps, a P-type ATPase and a membrane potential-dependent three-polypeptide cation/proton antiporter. Antibacterial experiment demonstrated significant results against J53pMG101, after incubation on 0.1wt% AgNP/NSP contianing agar plate. CFU (colony formation unit) of J53pMG101 remarkably decreased to below 10%. Nutrient uptake assay and intracellular energy level indicated that AgNP/NSP treated J53pMG101 cells will lose the ability to ingest glucose and further decrease ATP level inside the cells. ICP-MS results shown that the concentration of Ag^+ dissolved from 0.1wt% AgNP/NSP was at ppb level. Thus we speculate that the antibacterial mechanisms of AgNP/NSP might not be mainly contributed by Ag^+ dissolved from silver nanoparticles but through another approaches.

Keywords antibacterial; silver nanoparticles; silver resistance, J53pMG101, nanohybrid

Antibiofilm activity of nanosized magnesium fluoride

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The ability of bacteria to develop antibiotics resistances and colonize abiotic surfaces by forming biofilms is a major cause of medical implant-associated infections and results in prolonged hospitalization periods and patient mortality. This raises the urgent need to find novel approaches to inhibit bacterial colonization of surfaces. One approach comes from recent progress in nanotechnology, which offers an opportunity for the discovery of novel compounds with antimicrobial activity as well as the use of "nano-functionalization" surface techniques.

In this study, we present a first demonstration of the antibiofilm activity of metal fluoride nanomaterials. Using an unreported microwave-based synthesis of MgF_2 nanoparticles (MgF_2 Nps) in ionic liquid, we demonstrate their ability to inhibit biofilm development of common nosocomial biofilm-forming pathogens.

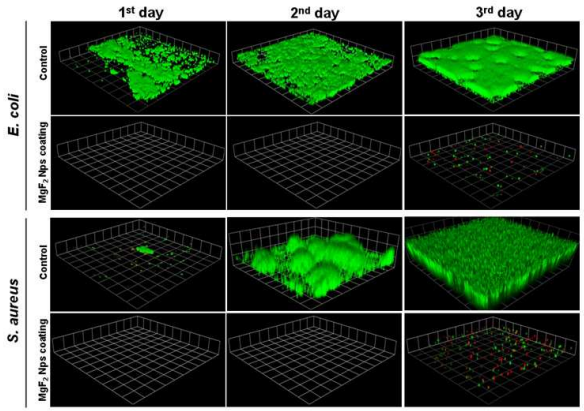
Scanning and transmission electron microscopic techniques indicated that the MgF_2 Nps attach and penetrate into the cells. Flow cytometry analysis revealed that the Nps caused a disruption in the membrane potential. The MgF_2 Nps also induced membrane lipid peroxidation and once internalized can interact with chromosomal DNA.

Based on these findings we further explored the possibility of using the MgF_2 Nps to coat surfaces and inhibit biofilm formation. A microwave synthesis and coating procedure was utilized to coat glass coupons. The MgF_2 coated surfaces effectively restricted biofilm formation of the tested bacteria.

This study emphasizes the potential of using metal fluoride nanoparticles as a new approach for the design of sterile surface coatings that may be useful for various medical applications.

Keywords: nanoparticles; magnesium fluoride; biofilms; antimicrobial properties, sterile surfaces

Antibiofilm activity of MgF_2 Nps coatings



Antimicrobial active films

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Consumer requests for fresh and minimally processed foodstuff are growing rapidly. On the other side, retailers and distributors are asking for food products that stay fresh and - especially microbiologically - safe for a very long time in order to get more logistic flexibility.

Therefore simple packaging with a passive barrier layer is no longer sufficient. For this reason, especially in the Japanese market, active packages have been in use for more than 20 years. Since 2009 there has been a new EU regulation on active and intelligent packages that facilitates the employment of this new packaging technology in the European Union.

At the Fraunhofer Institute of Process Engineering and Packaging, Germany, a new antimicrobial active film has been developed. The activity of this film is based on sorbic acid. Sorbic acid is a conventionally used preservative that represents no health or allergenic hazard. It is approved under food law for many different kinds of foods. This film releases very low amounts of sorbic acid to the surface of the packaged food. In this way it protects the food at the contact area from microbial contamination. Thus longer shelf life and safer food can be guaranteed. The common way of preserving the foodstuff by adding the preservative to the whole food matix can be avoided.

For producing this antimicrobial active film, sorbic acid is added to a polyvinyl acetate lacquer and applied on common food packaging films. This lacquer enables a controlled release of the active component and at the same time can be used as a sealing layer.

Crucial for application of these films is the antimicrobial activity against different strains of pathogen and non pathogen microorganisms. To test the antimicrobial effectiveness, we used the Japanese Industrial Standard test method (JIS Z 2801:2000).

The antimicrobial active films showed a germ reduction of up to 10^6 colony forming units (cfu). The spectra of activity extended over spoilage bacteria (*Pseudomonas fluorescens)* to yeasts (*Saccharomyces cerevisiae*) moulds (*Aspergillus niger)* and pathogen food infecting bacteria such as *E. coli* and *Staphylococcus aureus*.

Positive effects and shelf life extension on foods could also been demonstrated on the mould *Penicillium roqueforti*. *Penicillium roqueforti* from blue cheese can be carried onto the Gouda cheese surface e.g. by cutting instruments.

For this test pieces of Gouda cheese were inoculated with spores of *Penicillium roqueforti*, covered with an antimicrobial film and then stored at 23 °C.

Germation of the mould on the Gouda cheese covered with a blank film could be observed after 7 days, while the germation on the gouda piece covered with antimicrobial active film took 4 weeks.

Keywords active packaging; sorbic acid

Antimicrobial activity of silver doped silica and titania based nanoparticles: an *in situ* study of photosterilization performance

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The resistance to antibiotics and disinfectants has recently become a major issue, especially for nosocomial and prosthetic device related infections. This triggered a renewed search for antimicrobial materials that are safe and cost-effective. A number of nanomaterials, such as silver, silver-gold, silica, titania and zinc oxide has been found to exhibit excellent antimicrobial properties through diverse mechanisms. Of these, metallic nanoparticles such as nano-Ag have been commercially exploited in products that require antimicrobial activity. There have been an increasing levels of concerns raised from various consumer groups as regards to the safety of nano-silver. The primary mechanism of its anti-microbial activity derives from the leaching of silver ions, which can potentially be harmful for human health. In contrast, in Ag-doped oxide nanoparticles, Ag can be immobilised in more stable form thus minimising the risk of the leaching. There is currently an inadequate understanding of the mechanism of the antimicrobial performance of such nanomaterials. Here, we investigate sterilisation and photosterilisation properties of Ag-doped nanoparticles of silica and titania against *Escherichia coli* and *MRSA* and compare their antimicrobial activity with undoped nanoparticles. For this, we constructed a purpose built illuminator to enable the study of photocatalytic activity of such nanoparticles during the microbial tests *in* situ. These results were then compared against the photocatalytic activity of these nanoparticles, so as to understand the role of Ag.

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Antimicrobial activity of silver nanoparticles produced by γ-ray irradiation reduction

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Silver nanoparticles have been used as an effective antimicrobial agent for many decades, especially in the medical applications, such as biological implants, dressings and bandages for treatment of burns, wounds and several bacterial infections. The utilization of silver nanoparticles has also currently emerged up with diverse areas including household items, toys, clothing, food storage containers, face masks, laundry detergent, etc. Antimicrobial activity of the silver nanoparticles seems to be related to their dimension and shape. Extensive research has been conducted to synthesize silver nanoparticles with controllable shape, size, and size distribution. The reduction of silver salts by chemical reducing agent is though effective and simple, the biological toxicity and the environmental hazard of the residual reducing agent are problems. γ-Ray irradiation has been an alternative way to overcome those limitations and to eliminate the removal of reducing agent step. Therefore, the objective of the present research was to investigate the antimicrobial activity of the silver nanoparticles prepared by γ-ray irradiation reduction method and the utilization of the silver nanoparticles in fabrication of antibacterial film. Silver nanopartcles were prepared by a γ-ray irradiation reduction (dose ~ 25 kGy) of silver nitrate (0.04 mmol) in the presence of chitosan stabilizer (0.5% (w/v)). The obtained nanoparticles showed a characteristic surface plasmon band at 411 nm as well as a positively charged surface with a zeta potential of +40.4 mV. The nanoparticles were spherical shape with a mean diameter of 20-25 nm (Figure 1A). In addition, the silver nanoparticles dispersed in γ-ray irradiated chitosan solution also exhibited antimicrobial activity against *E. coli*, *S. aureus* and *B. cereus* with a minimum inhibitory concentration of 5.64 μg/mL. Chitosan-starch blend films containing silver nanoparticles were fabricated by a solution casting method. The incorporation of silver nanoparticles slightly improved tensile and oxygen gas barrier properties of the polysaccharide blend films, while deteriorated their water vapor barrier properties. Antimicrobial efficacy against *E. coli*, *S. aureus* and *B. cereus* of the films was enhanced by loading of silver nanoparticles (Figure 1B). The results suggest that the silver nanoparticles dispersed in γ-ray irradiated chitosan solution and the chitosan–starch blend films containing silver nanoparticles have great potential as antimicrobial agent and antimicrobial film that could be possible to use in medical and food packaging applications.

Keyword: silver nanoparticles; antimicrobial activity, γ-ray irradiation; chitosan; starch

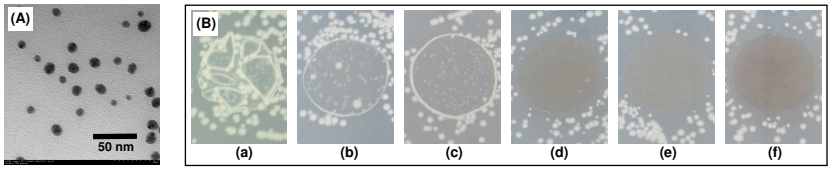

Figure 1 (A) TEM micrograph at 100 kV of silver nanoparticles prepared by γ-ray irradiation reduction in the present of chitosan solution and (B) inhibitory zone against *E. coli* after incubation at 37 °C for 24 h of (a) starch film, (b) chitosan film, (c) chitosan-starch blend film and (d)-(f) chitosan-starch blend films containing different contents of silver nanoparticles: (d) 0.78, (e) 1.53 and (f) 2.94 mg/g of polysaccharides.

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Antimicrobial efficacy of low-temperature plasma against 65 genetically characterized *Staphylococcus aureus* isolates including PVL-positive, MRSA and c-MRSA strains

Hübner NO; Matthes R; Gruman D; Holtfreter S; Bröker B; Koban I; Bender C; Kindel E and Kramer A

Aim Staphylococcus aureus is an important human commensal as well as pathogen. Low-temperature plasma is a new, promising, physical alternative to chemical means of antibacterial therapy, but data on the efficacy against different pathogens or different strains of one pathogen are sparse. We therefore tested the antimicrobial efficacy of low-temperature plasma against 65 different *Staphylococcus aureus* isolates including PVL-positive, MRSA and c-MRSA strains.

Method. Nasal-carriage isolates (n = 25), clinical isolates from furuncoloses patients (n = 17) and blood cultures (n = 20) as well as control strains (n=2) from the Robert-Koch Institute were tested for their antimicrobial resistance and genetically characterized. Serial dilutions of overnight cultures were plated on blood agar plates using an automated spiral plater (Meintrup DWS, Germany) and treated with low-temperature plasma from a HF-Plasma-Jet KinPen09 with a gas flow of 5 standard l/min of argon as carrier gas under room temperature. The plasma source was fixed in a computer-controlled x/y/z table. The entire surface of the test object was treated in spirals at a speed of 10 mm/s. Total contact time per point was 6 s. Argon gas alone was applied as a control.

Results. Average reduction against untreated control was 2.66 $\log_{10}$ [CI95: 2.60 - 2.72]. Susceptibility of isolates from different sites differed slightly with mean reduction factors of 2.57 [2.46 - 2.67] for nasal isolates, 2.7 [2.58 - 2.81] for blood cultures, 2.79 [2.69 - 2.88] for furuncoloses and 2.38 [-1.12 - 5.87] for controls. Neither methicillin-resistance nor presence of the PVL locus were associated with reduced susceptibility to plasma treatment ($p<0.05$).

Conclusion. In this pilot study, low-temperature plasma was highly effective against all tested strains. Further research should be done to evaluate this promising physical alternative to chemical means of antibacterial therapy.

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Antimicrobial films from quaternary ammonium compounds and poly(methylmethacrylate)

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Microbial adhesion and biofilm formation on medical devices represent a common occurrence that can lead to serious illness and even to patient death. Besides, surfaces may act as reservoirs of microbes which could lead to the spread of infection upon being touched by healthcare workers or patients.[1] Treatment of established biofilms with antimicrobial agents are difficult because the organisms are encased within a protecting microenvironment. Main approaches to reduce adhesion are based either on killing microbes upon contact or preventing adhesion without killing bacteria.[2] Cationic surfactants and lipids such as dioctadecyldimethylammonium bromide (DODAB) have been established as anti-infective agents. [3] Microbicidal coatings based on immobilization of the DODAB lipid in a polymeric network of poly(methylmethacrylate) (PMMA) yielded stable and homogeneous hybrid films on silicon wafers.[4] In this work these hybrid films containing quaternary ammonium compounds (QACs) such as DODAB or cetyltrimethylammonium bromide (CTAB) are further evaluated regarding physical properties and antimicrobial activity against two bacterial species of clinical interest which are particularly important in formation of biofilms, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The films were prepared by spin-coating of a chloroformic solution of DODAB or CTAB and PMMA on a glass coverslip. In this chloroformic solution, DODAB or CTAB concentrations ranged from 0.03 to 3.8 mM, which corresponds to 0.01875 to 2.4 mg/mL of DODAB or 0.01092 to 1.38 mg/mL of CTAB, whereas PMMA concentration was fixed at 10 mg/mL. After chloroform vaporization, the spin-coated films were characterized by determination of contact angle and surface tension as a function of QAC concentration. After one hour film-bacteria interaction, antimicrobial activity against *P. aeruginosa* and *S. aureus* was quantitatively evaluated from plating and CFU counting as previously described for other films.[5] Hybrid PMMA-QAC films showed higher wettability than pure PMMA with wettability increasing as a function of QAC concentration. Regarding the surface tension, for PMMA-DODAB films immersed in aqueous solutions, surface tension at the air-water interface remained constant and equal to the surface tension of water showing that DODAB remains mechanically imprisoned in the polymeric network and does not leave the film to occupy the air-water interface. For PMMA-CTAB films, immersed in aqueous solutions, surface tension at the air-water interface showed a slight reduction up to 60 mN/m, suggesting that CTAB diffuses more readily than DODAB from the polymeric network. The antimicrobial effect of these bybrid films was clearly dependent on QAC concentration. For PMMA-DODAB films, 0% cell viability was obtained for films prepared from 2.4 mg/mL QAC solution for both bacteria species tested. Regarding PMMA-CTAB films, 0% cell viability for *P. aeruginosa* and *S. aureus* was obtained from 0.7 and 0.07 mg/mL QAC solution, respectively.

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Keywords antimicrobial surfaces; hybrid films; cationic lipid; pathogenic bacteria.

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Antimicrobial packaging affects spoilage microbial populations and volatile organic compounds release in meat stored under vacuum

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An antimicrobial solution contaning nisin and other antimicrobials was used for the development of active pouches of plastic barrier film for meat packaging. Beef cuts of about 500 g were singly packed in antimicrobial and in non activated (control) plastic bags and stored at 1°C. Single samples for both antimicrobial stored and control bags were taken at different times up to 46 days of storage to monitor the spoilage associated bacterial populations. The icrobioa was investigated by using PCR-DGGE analysis after DNA extraction directly from beef. Meat colour was measured and volatile compounds were monitored in the headspace of meat samples by both SPME-GC/MS and a quartz crystal microbalances (QCM)-based electronic nose. The use of the active packaging showed its antimicrobial power from the start by retarding the growth of the populations of LAB, carnobacteria and *B. thermosphacta* for two weeks. The PCR-DGGE fingerprints did not show dramatic changes on the profiles of control and treated samples. After 36th days of storage the profiles of meat stored in active and non activated packagings were similar and showed the presence of bands identified as *Pseudomonas* spp., *C. divergens* and *Rahnella aquatilis*. A purplish-red colour characterized both control and treated samples. Different volatile organic compounds were detected during storage of meat such as alcohols, aldehydes, ketones and carboxylic acids; these can be related to microbial activities potentially leading to spoilage off-flavors. Metabolites produced in the highest quantities during storage were acetoin and some carboxylic acids that appeared in the early stages of storage. The microbial metabolic activity showed the influence of the use of the antimicrobial film particularly from 9 up to 36 days with a maximum in the differences of volatile metabolites in samples analyzed at 20 days. By contrast, the volatile profiles of control and treated samples were very similar after 46 days. The statistical analysis of e-nose data showed a clear differentiation between treated and control meat samples indicating a good potential of the e-nose for the differentiation of meats stored in different packaging conditions. In conclusion, the active packaging can be effective not only to reduce the loads of certain spoilage microbial populations but can also affect the diversity of metabolite release in the headspace of meat with a clear impact on meat quality.

This study was partly supported by a EU project (SYMBIOSIS-EU) within the 7th Framework Programme (ref. Grant agreement N°. 211638).

Keywords bacteriocin-activated antimicrobial packaging; meat spoilage

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Antimicrobial Polymers: Current and Future Perspectives

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Microbial infection remains one of the most serious complications in several areas. Particularly in medical devices, drugs, health care and hygienic applications, water purification systems, hospital and dental surgery equipment, textiles, food packaging and food storage. Antimicrobials gain interest from both the academic research and industry due to their potential to provide quality and safety benefits to many materials. However, low molecular weight antimicrobial agents suffer from so many disadvantages such as toxicity to the environment, and short-term antimicrobial ability. To overcome problems associated with the low molecular weight antimicrobial agents, they are prepared by introducing antimicrobial functional groups into the polymer molecules. The use of antimicrobial polymers offers promise for enhancing the efficacy of some existing antimicrobial agents and minimizing the environmental problems accompanying conventional antimicrobial agents by reducing the residual toxicity of the agents, increasing their efficiency and selectivity, and prolonging the lifetime of the antimicrobial agents. Research concerning the development of antimicrobial polymer represents a great a challenge for both academic world and industry. This lecture reviews the state of the art of the antimicrobial polymers. In particular, it is discussing the requirements of antimicrobial polymers, factors affecting the antimicrobial activities, methods of synthesizing antimicrobial polymers, major fields of applications and future and perspectives in the field of antimicrobial polymers. Special attention will be made to antimicrobial would dressing.

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Antimicrobial Zinc Complex Design for Specific Functions; Wet Polymer vs. Plasma Polymer

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Metal ions that have antimicrobial properties such as silver, copper and zinc have been utilized to make novel functionalized compounds. Design of metal complexes allows us to make new model systems for incorporating antibacterial metal ions into polymer systems. Two polymer systems; hydrogel type polymers, and plasma polymers, both with different physical properties have been functionalized with zinc ions. Zinc(2,2'-Bipyridine-(mono-2-(Methacryloyloxy)ethyl succinate)$_2$) or Zn(Bipy-(MMOES)$_2$) a zinc carboxylate monomer was designed to mimic commercial crosslinkers and polymerised using AIBN initiated free radical polymerisation to give a hydrogel type polymer. The Zn(Bipy-(MMOES$_2$)) monomer was then copolymerised with acrylic acid to show how it can be incorporated into polymer networks. The polymers were shown to be pH responsive and release zinc ions at lower pH and to have antibacterial activity against both gram positive *Methicillin susceptible staphylococcus aureus* (MSSA476) and gram negative *Pseudomonas aeruginosa* (PA01).

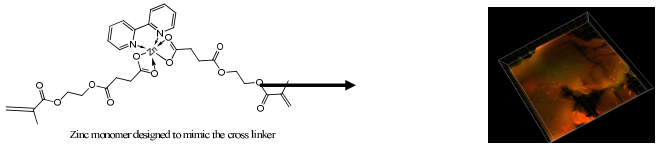

Fig 1. Zinc monomer design and confocal image using live/dead staining on polymer

Zinc Schiff base complex have synthesised for the purpose of being used in plasma polymerisation to create antimicrobial thin films on substrates such as non-woven fabric and polystyrene.

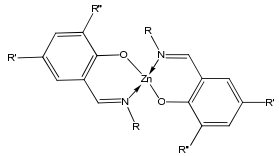

Fig. 2 Structure of zinc Schiff base complexes for plasma polymerisation (R'= H, OMe)

By altering the structure of the Schiff base ligand the structure vs. antimicrobial efficacy relationship has been analysed my comparison of MIC$_{100}$ against both *Methicillin* susceptible *staphylococcus aureus* (MSSA476) and *Pseudomonas aeruginosa* (PA01). Zinc Schiff base complex has been designed for use as a grafting to approach.

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Apoptosis-like of pathogenic fungi in host tissues as a community survival strategy

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We have reported recently that lactoferrin, an innate defense protein present in the mucosal fluids, induce an apoptotic-like process in the pathogenic yeast *Candida albicans* [1]. Although apoptosis in Metazoa has been associated with normal tissue homeostasis, the functional role of apoptosis-like in fungal species has yet be fully defined [2]. What would the advantage be for *C. albicans* cells to undergo apoptosis in a host-microbe interaction context? We speculate that yeast apoptosis mimics host cell death processes to avoid the eradication of the overall fungal community. The "silent death" (apoptosis) of *C. albicans* cells could be advantageous respect to cell lysis (necrosis) because the absence of cytoplasmic material released could delay, attenuate, or not trigger a defense response (i.e., inflammatory response) against other clonal relatives. This presumed scenario could allow some yeast cells to survive, hence contributing to the yeast community´s long term survival on healthy host mucoses as commensals, or in damaged tissues as opportunistic pathogens. Furthermore, the *C. albicans* apoptosis-like process may be related to its colonizing and/or immune- evasion abilities rather than its pathogenicity because other non-pathogenic yeast (i.e., *S. cerevisiae*) are also capable of undergoing apoptosis. Although future studies should focus on validating this hypothesis, the apoptosis-like process witnessed in *C. albicans* cells could represent an adaptive response of fungal apoptotic mechanisms to certain lethal stimuli, such as the one induced by lactoferrin, mimicking the host cell apoptosis. This survival strategy could extend the life span of the overall yeast population on host mucoses.

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Keywords: apoptosis, *Candida albicans*

Assessing the antimicrobial efficacy of in-package cold plasma treatments; development of an experimental protocol

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Disinfection is a process widely applied in food and pharmaceutical industry in order to achieve the removal of microorganisms from an inanimate surface. These microorganisms may include pathogens and must be reduced to a level that is not harmful to human health or have an adverse effect on the quality of perishable goods. Currently many novel methods of disinfection are under research including the use of plasma treatments. These treatments use electrical plasma to remove, kill or inactivate microorganisms directly or indirectly by the activity of the generated radical species. Plasma is a type of ionised electrically neutral gas which contains electrons, ions and neutrals in excited states.

PK-1 device is a novel plasma generator which consists of a dielectric barrier discharge system. The PK-1 device has demonstrated high potential for inactivating microbes at in-package conditions. This study was carried out to investigate the antimicrobial efficacy of the PK-1 system and to diagnose the active species produced by this system. The inactivation of the indicator *Listeria innocua* was assessed in an in-package system using an agar based medium. The plasma emission was captured via a 0.22 NA optical fibre and analyzed using a low-resolution UV-vis spectrometer operated in the wavelength range 200 nm – 1,000 nm.

Samples were prepared using inanimate surfaces inoculated with *Listeria innocua* which were then placed in plastic resealable bags, filled with air/helium mixed gas and treated with plasma for 5 minutes. Agar-agar was selected as the surface media for the plasma treatment. The rate of bacterial growth on this media was limited when compared with that of nutrient agar over a 24 hours period following the plasma treatment. The optical emmision spectrum was composed of an entirely discrete structure comprising nine intense lines in the wavelength range 320 nm to 400 nm. The spectral features observed can be partly attributed to emission bands in the nitrogen molecule and the nitrogen molecular ion N_2^+. Strong emission lines from various excited states of the atomic species O, O^+, N and N^+ were also found in the same spectral range. The plasma treated samples were maintained at room temperature for up to 24 hours to determine if antimicrobial activity due to the residual effect of the produced active species was evident. Kinetic samples and untreated controls were processed and colony forming unit measurements were obtained. The results of the treated samples were compared as ratios with each of the separate control samples. From this study it was found that a 2 log reduction of *Listeria innocua* was possible using air/helium gas with a majority of the microbial inactivation occurring within 1.5 hours post-treatment. This study has proved that the PK-1 device can be applied as an antimicrobial technique having a post treatment effect for in-package products.

Keywords *Listeria*; plasma;

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Assessment of surfaces containing silver for the application in household refrigerators

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In perishable food supply chains, from primary production to retail, several rules and regulations exist to ensure high quality and safe products. From the point of sale, the end consumers themselves have the responsibility for the hygienic handling and storage of chilled food products. Besides incorrect storage temperatures, cross-contamination is an area of great concern in private households. Bacteria can be transferred via hands, the air and stored food to refrigerator surfaces. Refrigerator manufactures try to support consumers by adding silver additives into refrigerator inner liners. However, it is unclear, whether these surfaces are able to decrease contamination under the special application conditions within refrigerators.

Thus, the aim of this study was the investigation of surfaces containing silver in terms of their ability to reduce surface bacteria within domestic refrigerators.

The antimicrobial activities of surfaces containing silver were analyzed by adapting the test method JIS Z 2801 (2000) to the special application conditions within refrigerators to investigate the ability of these surfaces to reduce surface bacteria. The influence of temperature, time, bacterial strain, food residuals and the composition of different materials on the rate of antimicrobial activity has been investigated. Based on the results, an evaluation scheme for the assessment of antimicrobial surfaces for the special purpose of food contact surfaces has been developed.

It became evident that the tested silver containing surfaces are in general able to reduce surface bacteria, but the antimicrobial activity strongly depends on environmental parameters. Low temperature conditions (5°C) decreases the rate of activity. Also the individual sensibility of bacterial strains has a strong influence on the rate of antimicrobial activity. Thus, the reduction rate of *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Lactobacillus delbrueckii* varied between $\log_{10}$ 1.0 and 7.8 cfu/ml. Besides that, protein or starch residuals on surfaces decreased or completely inhibited the activity of silver. Furthermore, the silver concentration and the basic polymer influences the silver release rate thus the rate of antimicrobial activity. This means the application of silver additives in refrigerator surfaces delivers only an additional benefit, if the surfaces are free of protein and starch residuals. The rate of antimicrobial activity is thereby strongly influenced by the described factors (bacterial strain, temperature, composition of material, etc.).

The multifarious influencing factors on the antimicrobial activity and the special regulations in the food chain showed the basic need for the evaluation of antimicrobial surfaces with regard to the planned application. Hence, the developed scheme allows a more precise assessment of the effectiveness of the surface with regard to the application. Within this scheme, the factors microorganisms, environment and antimicrobial agent are considered.

The increasing usage of silver additives during the last years in several applications of daily live also bears risks due to the increasing entrance of silver ions into the environment (e.g. entry of silver ions into sewage), the migration of silver into food products and the promotion of resistances against silver. Thus, a more careful use of silver surfaces is advisable with regard to sustainability. The developed scheme delivers an important contribution for the assessment of surface with regard the planed application. Through the adapted testing, application of silver additives in areas, where the additive is not active can be avoided.

Keywords surfaces containing silver, food, refrigerator, influencing factors on antimicrobial activity, evaluation scheme

Bacteria entrapment coatings for smart food packaging

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Food contamination is the cause of many health and economic problems especially for the presence of psychrotrophic pathogens and spoilage microorganisms. If bacterial growth could be delayed or inhibited it would be possible to obtain a great advantage relatively to the public health and the shelf-life of products.

Recent developments in food preservation have been focused on the use of natural antimicrobial substances. A new methodology proposed to reduce the bacterial growth in foods is the use of active packaging and one possible approach is the application of functional coatings to the substrates typically used for food packaging.

Here we report a study about the preparation and the antibacterial activity comparison of two different type of functional coating films including either living *Enterococcus casseliflavus* IM 416K1 bacteriocin-producer or Enterocin 416K1, entrapped in a PVOH-based hybrid coating applied to PET films. The methods employed for the bacteria entrapment allowed them to survive within the coating even after one month at 4°C and the strain was able to grow and continuously produce bacteriocin when in contact with a nutrient. A quite homogeneous distribution within the hybrid was confirmed using microscopy techniques (fig. 1).

The antimicrobial activity of the coated films was evaluated against *Listeria monocytogenes* NCTC 10888, one of the most common food-borne pathogen, by qualitative modified agar diffusion assay (fig. 2: live-enterococcus doped film (1), enterocin doped film (2) and concentrate Enterocin 416K1 (3)) and by direct contact with artificially contaminated food samples stored at room and refrigeration temperatures. The qualitative evaluation assay demonstrated a remarkable anti-listeria activity of the coatings and the inhibitory capability was confirmed also in the artificially contaminated food samples. In particular, live-enterococcus doped film demonstrated a better anti-listerial activity with respect to enterocin doped film, highlighting the capacity to assure a long lasting efficiency.

These results suggest that the incorporation of living bacteriocin-producers into films for food packaging may be an interesting development, which allows to combine the functions of anti-microbials with the protective functions of the packaging. In particular it has to be emphasized that coatings doped with live-enterococcus can behave like smart coatings as they are able to be responsive to an increased growth rate of *Listeria monocytogenes*, induced by an increase of the temperature, by producing bacteriocin at a faster rate and resulting in a high antibacterial efficiency.

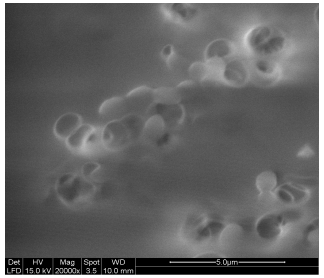

Fig. 1

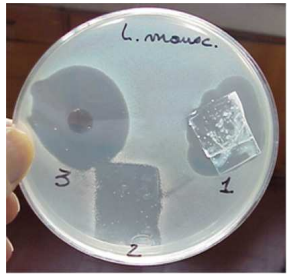

Fig. 2

Keywords antibacterial coatings; active food packaging; bacteria entrapment

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Bacteriophages actions on *Salmonella* Enteritidis biofilm

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Infections caused by Salmonella spp. are responsible for the high incidence of diarrheal diseases in humans and constitute a public health problem. The consumption of contaminated foods of animal origin, especially eggs, meat, dairy products, are the main source of salmonellosis in humans. Like other microrganisms, *Salmonella* sp. may be involved in processes of adhesion and biofilm formation on surfaces of equipment and utensils in the food industry in which it may compromise the microbiological quality of food and cause health risk to consumers. Studies show the potential application of bacteriophages in the control of *Escherichia coli* biofilms on coupons of PVC [1], biofilms of *Pseudomonas aeruginosa*, *Acinetobacter johnsonii* and *Bacillus subtilis* in ultrafiltration membranes for wastewater treatment [2] and reduction of *P. fluorescens* on stainless steel coupons [3]. The objective of this work was to produce biofilms of *Salmonella* Enteritidis on stainless steel coupons and evaluate its reduction by the use of a pool of lytic bacteriophages. A Box-Behnken design in three factors was used with three concentrations of bacteriophages (10^5, 10^7 and 10^9 PFU.mL^{-1}), three contact times (10, 35 and 60 min) and biofilm of three ages (4, 8 , 12 days) with three replications at the central point. To promote biofilm formation, the coupons of stainless steel (AISI 304) were immersed in TSB diluted stock (1/20) added with 10% *Salmonella* Enteritidis inoculum (ATCC 13076) and incubated at 25°C. At each sampling time the coupons were washed in a 0.85% saline solution for the removal of planktonic cells and subjected to treatments with the pool of bacteriophages. After the contact times of each treatment, the coupons were washed again in a 0.85% saline solution for the removal of bacteriophages and dead cells and subjected to ultrasound for 30 min to remove sessile cells. The count of *Salmonella* sp. was performed using the microdroplets technique in TSA agar after incubation at 37 ° C for 12 hours. This procedure was repeated for the coupons with biofilms of 8 and 12 days. The statistical analysis were performed in the version 14 of the MINITAB ® Statistical Software program. The coupons were visualized by electronic scanning microscopy (LEO1430VP) after the treatment with bacteriophages. It was observed that there was an increase in the number of cells in the biofilm according to the time of cultivation, the count of *Salmonella* sp. was 6.1 log CFU cm^{-2} to 4 days, 6.9 log CFU cm^{-2} to 8 days and 7.7 log CFU cm^{-2} with 12 days. The reduction of the *Salmonella* biofilm observed in the stainless steel coupons following the Y = - 0.59 to 0.15. A + 0.57. B - 0.02. C -0.03. B2 + 0.0035. AC model, where A (concentration of bacteriophages), B (age of the biofilm) and C (contact time). The largest reductions were observed in the treatments with 10^7 PFU.mL^{-1} bacteriophages, 35 min contact in biofilms with 8 days of age. In the microscopic evaluation it was observed a reduction of adherent cells treated with bacteriophage at concentrations of 10^9 and 10^7 PFU.mL^{-1} and little reduction of the treatment with 10^5 PFU.mL^{-1} compared with the control (Figure 1). The infection of the biofilm cells by bacteriophages is highly dependent on the chemical composition and environmental factors such as temperature, phase and growth mediums and concentration of bacteriophages. It can be concluded that the used bacteriophages were able to reduce *Salmonella* Enteritidis biofilms in stainless steel coupons of different ages, and constitute an alternative to control this pathogen in the food industry.

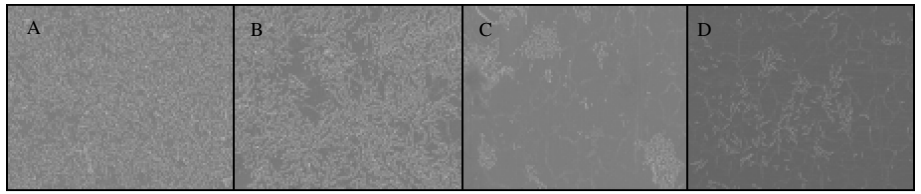

Figure 1. Changes in biofilm formed in stainless steel coupons after a bacteriophages pool treatment. [A (control), B (10^5 PFU.mL^{-1} for 35 min), C (10^7 PFU.mL^{-1} for 35 min) and D (10^9 PFU.mL^{-1} for 35 min).]

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Keywords: Bacteriophages, biofilm, *Salmonella* spp.

Biocompatibility and Antibacterial property of Cold Sprayed ZnO/Titanium Composite Coating

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The deposition using cold gas dynamic spraying of Zinc oxide/Titanium (ZnO/Ti) composite have been fabricated on Al 6061 substrate, their biocompatibility and antibacterial properties were studied. RAW 264.7 cell was used for the biocompatibility test while Escherichia coli (DH5α) was employed in antibacterial study. The ratios of ZnO to Ti in their composite powders were 20:80, 50:50 and 80:20 (weight %). The ZnO/Ti coatings were successfully deposited using cold spraying parameters of 13-15 Bars under helium gas, temperature of between 300-400^{o}C. The results obtained showed that the ZnO/Ti coatings exhibited significant antibacterial effects against E. coli. For biocompatibility results, RAW cells spread and adhered well on the coating samples especially on low concentration of ZnO contained (ZnO20/Ti80).

Keywords; Cold spray, Zinc oxide, Titanium, Biocompatibility, Antibacterial property

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Biofilm formation on silicone materials containing various antimicrobial agents

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The colonisation of microorganisms and subsequent biofilm formation on the surface of polymeric high voltage insulators affect the surface properties and can lead to failure of the insulators. In this study, silicone materials were prepared with different antimicrobial agents. The materials were analysed for the changes in the physical, chemical, surface and mechanical properties before and after biological growth test.

Microorganisms used for the biological tests were fungi defined in the international standard test ISO 846 for materials for electrical applications (*Aspergillus niger van Tieghem, Penicillium funiculosum Thom, Paecilomyces variotii Bainier, Chaetomium globosum Kunze: Fries, Aspergillus terreus Thom, Aureobasidium pullulans (de Bary) Arnaud & Penicillium ochrochloron Biourge)* and algae isolated from insulators in Sri Lanka and Tanzania (*Chlorella vulgaris var. Autotrophica + various bacterial strains*). Fungi growth test was performed by inoculation of the fungi on the surface of the materials and incubation in an oven at 28°C and 98% humidity for a specific period. Algae growth test was performed by inoculation on the material surface and subsequent incubation in room temperature under constant fluorescent lamps for a specific period.

The results indicated that some of the samples could prevent the biofilm formation on the surface of the materials while the microbial growth was unaffected on the pure silicone rubber.

Keywords: High voltage insulators, silicone rubber, biofilm, biological growth, biocide

bioFILM-PA™ : A standardised method for antimicrobial susceptibility testing of planktonic and sessile *Pseudomonas aeruginosa* isolated from cystic fibrosis patients.

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Objectives: In cystic fibrosis (CF) patients, there is strong evidence that *P. aeruginosa* also exists in the biofilm form on mucosal surfaces, and that the routine anti-microbial testing in broth culture may not satisfactorily evaluate this state. bioFILM-PA™ assesses the minimal bacterial eradication concentration (MBEC) of single or combined antimicrobial agents against *P. aeruginosa* existing in both planktonic and biofilm forms. We have defined the quality parameters for performance of the test, and we have done a retrospective clinical evaluation of the results of this laboratory technique in a number of CF patients.

Methods: For accuracy and reproducibility, *P. aeruginosa* ATCC 27853 was tested a total of 39 times in two laboratories by different technologists. Biofilms were prepared in tryptic soy broth on plastic pegs in a 96 well format and placed into trays containing Mueller-Hinton broth with antimicrobial agents at varying concentrations, both alone and in combination. After overnight incubation at 35^0C, the pegs were washed, and placed in a recovery medium to measure inhibition of the biofilm cells. Results were recorded as susceptible (S), Intermediate (I), or resistant (R) according to CLSI systemic breakpoints. Reproducibility was calculated by dividing the total number of results that fell within one well of the mode, by the total number of results. For clinical analysis 46 different strains from 19 CF patients between the ages of 8 to 51 years were included. Spirometry was used to estimate the degree of airflow obstruction.

Results: Reproducibility of MIC for 1375 evaluable tests was 99% for both laboratories combined. For biofilm susceptibility, reproducibility for 468 evaluable tests using CLSI breakpoints for planktonic cultures (no biofilm ranges) was 96.2%. Reproducibility of MIC of combinations of antimicrobials for 1365 test results in the biofilm-PA™ device was 98.5%. Reproducibility of 35 combinations of antimicrobials in the biofilm susceptibility test varied depending on the combination of agents. Among the clinical isolates, 39 of the 46 strains showed increased resistance in the biofilm forms compared to planktonic cells, ranging from 1 to 47 loci of differences with a mean at 19.3 and a standard deviation at 14.1. Spirometry was available pre-culture in 14 patients. One was unobstructed, 9 had severe and 4 had mild or moderate airflow obstruction The number of resistant biofilm and planktonic strains was greatest in those with severe airflow obstruction (Anova, p=0.002). Antimicrobial treatment changes were made in 6 patients based on MBEC results.

Conclusions: bioFILM-PA™ provided a high level of reproducibility for single (>95%) and combination (98.5%) agents against the quality control strain *P. aeruginosa* ATCC 27853. Our studies document a clear distinction in resistance profiles between planktonic and biofilm forms of *P. aeruginosa* from CF patients and that the severity of airflow obstruction correlated best to the extent of resistance in the biofilm form. These observations indicate that bioFILM-PA™ may be used successfully to test clinical isolates of *P. aeruginosa* for planktonic and sessile susceptibility against agents that may be effective for therapy. Prospective studies with larger patient sample size are required to validate the clinical utility of this laboratory tool.

Keywords: Biofilm susceptibility; P. aeruginosa

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Ceragenin CSA-13 and cholesterol exhibit antimicrobial activities against *Streptococcus pneumoniae* and other pathogenic streptococci

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The alarming increase of multidrug resistance worldwide has prompted a renewed interest in the development of new antibacterial drugs. In particular, the widespread increase of pneumococcal strains resistant to β-lactams and other antibiotics urges the development of preventive approaches and/or new therapies to efficiently fight this human pathogen. *Streptococcus pneumoniae* is the main bacterial pathogen responsible of serious infections such as pneumonia, sepsis and meningitis. Ceragenins, also termed CSAs (for 'cationic steroid antibiotics'), are facially amphiphilic compounds designed to mimic the activities of antimicrobial peptides and have been generated from a steroid scaffold. Several of these derivatives exhibit antimicrobial activity against a broad range of bacteria; they can incorporate stably into membranes forming complexes with phospholipids and are resistant to cleavage by proteolytic enzymes as compared to endogenous antimicrobial peptides. In this study, we examined the antimicrobial activities of the most potent analog, CSA-13, and cholesterol against *S. pneumoniae* and other pathogenic streptococci.

CSA-13 displayed potent antimicrobial activity against all the streptococcal strains tested with minimal inhibitory concentrations (MIC) of 1-2 μg ml^{-1}. CSA-13 triggered pneumococcal autolysis at concentration higher than 5 μg ml^{-1}. Bactericidal activity was also observed in cultures of other streptococci. The autolysis caused in pneumococcal cultures by CSA-13 was due by the triggering of the LytA amidase. 'Curing' of a *lytA* mutant by externally adding purified LytA prior to the addition of CSA-13 restored the lytic action of the drug. In addition, CSA-13 disaggregated pneumococcal biofilms, although at concentrations higher than those required for bactericidal activity against planktonic bacteria. Since CSA-13 has a low hemolytic activity, it is conceivable that this drug could be useful to combat streptococcal infections.

The cholic acid is one of the two major bile acids that are made in the liver by the cytochrome P450-mediated oxidation of cholesterol. We also showed that cholesterol in a soluble form exhibited bacteriolytic activity when added to pneumococcal cultures, including multidrug-resistant *S. pneumoniae*. Conceivably, cholesterol releases the lipoteichoic acid from the membrane in a manner similar to that of sodium deoxycholate, allowing LytA to degrade the cell wall peptidoglycan. Experiments of phenotypic curing also showed that the addition of cholesterol (25 μg ml^{-1}) was sufficient to restore the lytic effect of this sterol on cultures of LytA-deficient pneumococcal mutants. Besides, cholesterol displayed bactericidal activity on pneumococcal biofilms.

Keywords antimicrobial activity; ceragenin CSA-13; cholesterol; biofilms; streptococci

Characterizing the impact of antimicrobial agent exposure on adherence and biofilm formation by *Chryseobacterium* spp. isolates

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Chryseobacterium spp., while being isolated predominantly from the environment, are often associated with biofilms in clinical or industrial settings. In the aquaculture setting, *Chryseobacterium* spp. are regarded as spoilage organisms or opportunistic pathogens. Their ability to form biofilms would enable them to cause recurrent outbreaks of disease in aquaculture systems and/or increase the difficulty in controlling an outbreak due to the increased resistance phenotype displayed by biofilm-associated cells. The minimum inhibitory concentration (MIC) and minimum biofilm inhibitory concentration (MBIC) of 10 *Chryseobacterium* spp. isolates from fish were thus determined using the broth microdilution and modified microtiter plate assays, respectively. Eleven two-fold dilutions of five antimicrobial agents (azithromycin, ceftazidime, chloramphenicol, gentamicin and tetracycline), ranging from 0.008 - 128 µg/ml were used. The effect of MIC, sub-MIC and supra-MIC exposure of five antimicrobial agents at the time of adhesion and after 24 h biofilm formation was determined using modified microtitre plate assays. MBICs were 1 – 5 fold higher compared to the MICs of planktonic cells. However, these increases appeared to be both isolate-specific and antimicrobial agent-specific. MIC, sub-MIC and supra-MIC exposure at the time of adhesion caused significant decreases in bacterial adherence. However, 0.5 MIC exposure resulted in less significant decrease in adhesion. Isolate CH5, a *Chryseobacterium indologenes* strain, displayed increased adherence following 0.5 MIC exposure to all five antimicrobial agents. Similarly, exposure of 24 h biofilms to MIC, sub-MIC and supra-MIC resulted in increased detachment of cells from the pre-formed biofilm. Isolate CH2B, a *Chryseobacterium meningosepticum* strain, however displayed statistically significant increases in adherence for all five antimicrobial agents tested at MIC, sub-MIC and supra-MIC. Other isolates showed increased adherence with 0.5 MIC exposure to azithromycin and/or gentamicin. The degree of detachment for each of the antimicrobial agents was isolate-specific. Exposure of *Chryseobacterium* spp. isolates to antimicrobial agents at the time of adhesion is more effective in decreasing biofilm formation than attempting to break down mature *Chryseobacterium* spp. biofilms using antimicrobial agent exposure.

Keywords: *Chryseobacterium* spp., biofilm, sub-inhibitory antimicrobial agent

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Ciprofloxacin susceptibility patterns of planktonic and sessile *S. aureus, E. coli*, and *P. aeruginosa* – effect of the exposure time

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Several aspects of human infections within a clinical arena are related to biofilm development. Various surfaces, like indwelling devices and medical equipments, are prone to biofilm formation, causing subsequent pathogenesis. The application of antimicrobial agents is one of the main strategies to eradicate biofilms. However, the action of antibiotics could be inefficient duo to the high tolerance of biofilmes comparatively to the bacteria in planktonic state. Thus, it is of upmost importance the use of suitable antimicrobials with high efficacies to eradicate biofilms and to control nosocomial infections. Ciprofloxacin (CIP) is a broad spectrum fluoroquinolone antibiotic, often use against both Gram-positive and Gram-negative bacteria, that causes inhibition of bacterial cell division by the inhibition of DNA gyrase and topoisomerase IV.

With this study it was aimed to analyze the antimicrobial efficacy of CIP against *S. aureus*, *E. coli* K-12, and *P. aeruginosa* PAO1, in planktonic cultures and in biofilm state, and also to characterize the time–kill kinetics of ciprofloxacin in pre-established 1-day-old biofilms.

In planktonic cultures, the CIP susceptibility patterns were achieved by the broth microdilution method and by the determination of the number of viable cells. In sessile bacteria, the anti-biofilm activity of CIP was assessed using a standardized biofilm assay, quantifying the biofilm mass, through crystal violet, the respiratory activity, using the XTT reduction assay, and the number of viable cells. The time-kill kinetics of CIP were attained exposing 24-hour-old biofilms of each bacteria to ciprofloxacin (6 ug/ml) over time (until 24 h), and determining biofilm metabolic activity, biomass and cellular viability at regular time intervals.

Ciprofloxacin displayed dose-dependent activity against both bacteria in planktonic and biofilm states. The MIC values ranged between 0.185 ug/ml for *S. aureus*, 0.5 ug/ml for *E. coli* and 0.75 ug/ml for *P. aeruginosa*. These values revealed that it was needed a 4-fold increase in CIP dose to cause growth inhibition of Gram- bacteria comparatively to the Gram+ strain. The presence of CIP during biofilm formation did not kill totally the biofilm-associated cells neither eradicated the biomass adhered. CIP exhibited only a bacteriostatic effect for all strains studied emphasizing that this antibiotic is more effective on suspended than on biofilm cells.

The application of a constant dose of 6 ug/ml of CIP against pre-formed biofilms revealed an evident time-dependent effect in the antibiotic action, since a gradual reduction of biofilm activity, biomass and cell number occurred over time. However, the time of CIP exposure needed to reduce the biofilm characteristics varied from each bacterial species. In fact, the antibacterial effect was evident after 4 h of exposure of the antibiotic with the *S. aureus*, around 8 h of exposure with the *E. coli* and 6 h of exposure with the *P. aeruginosa* pre-formed biofilms. Once more, ciprofloxacin only presented bacteriostatical activity against all the bacterial biofilms, even when the exposure time reached 24 h.

In the range of experimental concentrations and exposure times tested, CIP didn´t reveal to be effective against bacteria cells neither in the pre-established biofilm nor during the process of biofilm development, in spite of the improved reduction of the metabolic activity, biofilm biomass and in the number of viable cells of *S. aureus*, *E. coli* and *P. aeruginosa* biofilms. Alternative treatments or combination of antibiotic therapies should be studied for the implementation of more effective ways of eradication biofilm-associated infections.

Keywords Biofilms; Planktonic Growth; Fluroquinolones; Ciprofloxacin; Exposure Time; Susceptibility patterns

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Coating a large variety of surfaces by AntiBacterial, Anti Viral, Anti biofilms and Antifungai Coatings on Textiles and Glasses Employing the sonochemical method

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In my presentation I'll introduce the sonochemical method as a technique for the deposition of nanoparticles (NP)on solid surfaces.I'll concentrate on the deposition of antibacterial nanoparticles on various textiles (cotton, nylon, polyester, and nonwoven). We are using either a one-step or a two-step procedure for the coating process. In the one-step process, we are fabricating the nanoparticles sonochemically, and subsequent to its creation it is pushed towards the substrate by fast moving microjets. The NP are embedded strongly in the substrate, and in glass the nanoparticles could penetrate as deep as 60 nm into the glass. When PMMA 2 mm beads were coated by silver NP we could find a few that have penetrated the polymer as deep as 1 mm.

Our first studies used Silver NP as the antibacteria coating. Silver is known for generations as antibacterial, and indeed the Ag NPs have killed the gram-negative *E. Coli* (strain 1313) as well as the gram-positive *Staphylococus aureus* (strain 195) bacteria very efficiently. Lately, since the FDA shows less enthusiasm towards Ag we have moved to NP of ZnO, CuO and MgO as antibacterial agents. They were coated on the above-mentioned fabrics and showed excellent antibacterial properties.

A special attention was dedicated to the question whether the NPs are leaching off the fabric when washed repeatedly. Leaching of the NP might be a threat to the environment. Lately the coated ZnO NP on cotton underwent 40 washing cycles at 90 ^{0}C in water in a Hospital washing machine, and no NP were found in the washing solution. Our vision is that all the textiles in the Hospitals of the future will coated by antibacterial NP. A detailed mechanism of the way the ZnO kills the bacteria will be presented.

MgF2 NPs coated on glass have demonstrated Antobiofilm Acitivity. I will demonstrate the bactericidal ability of these new fluoride nanomaterials to restrict bacterial colonization of two common biofilm pathogens. We prove the affecting interactions with MgF_2 Nps on bacterial cells by mechanical, potential damages to the membrane and the possibility to interact with nucleic acids. In Figure 1 we depict the effect of the MgF2 NP coated on glass on the growth of biofilms.

Keywords Sonochemistry, nanoparticles, antibacterial, antibiofilm

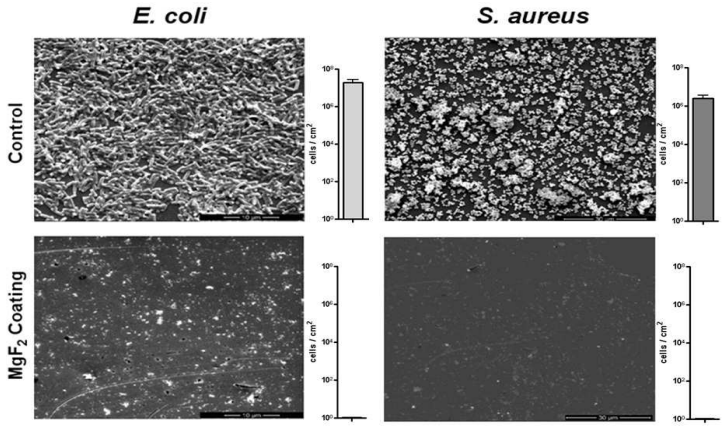

Figure 1. MgF_2 coated glass surfaces inhibit bacterial biofilm formation. SEM images and cells viability of *E. coli* and *S. aureus* biofilms grown on uncoated and coated glass surface.

Combined role of Ag nanoparticles and surface plasma treatment on PLGA nanocomposite antibacterial activity

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Nanoparticles of noble metals have been studied with growing interest, and silver (Ag) has been known to have a disinfecting effect and has found applications in traditional medicines. Several salts of silver and their derivatives are commercially employed as antimicrobial agents while Ag nanoparticles have aptly been investigated for their antibacterial property. Moreover, embedding of nano-sized metals into biodegradable polymer matrices represents a valid solution and permits a controlled antibacterial effect.

Biodegradable polyester including poly(lactide-coglycolide) (PLGA) with various lactide/glycolide ratios is involved in medical application while gas plasma treatment is extensively used for surface chemical modification of poly (lactide-co-glycolide) (PLGA) in order to increase the polyester hydrophilicity and to improve surface biological activity. Furthermore physicochemical properties of the nanocomposite surface are know to affect the rate of the initial bacterial adhesion and the subsequent biofilm formation.

The purpose of this study is to develop a novel biodegradable material with antibacterial properties and tuneable bacteria adhesion. The effect of Ag nanoparticles and the oxygen plasma treatment on the surface properties of PLGA, including hydrophilicity, surface chemical composition and morphology was investigated.

PLGA nanocomposite films, produced by solvent casting technique adding 1wt% and 7wt% of commercial silver nanoparticles, were investigated. The PLGA films and PLGA/Ag nanocomposite surface were treated by means of a radiofrequency plasma-enhanced chemical vapour deposition method, under oxygen flow. The surface of PLGA and PLGA nanocomposite films were characterized by field emission scanning electron microscopy (FESEM), atomic force microscopy (AFM), static contact angle (CA), and high resolution X-ray photoelectron spectroscopy (XPS). Antibacterial test and bacteria adhesion were performed *by Escherichia coli* RB and *S. aureus* 8325-4.

AFM investigations underline that the oxygen surface treatments induced an increase in roughness, mainly in the nanocomposites. The PLGA surface became hydrophilic after the oxygen treatment and its roughness increased with the treatment time. These surface effects had a dominant influence on the bacteria adhesion and growth. Oxygen treated PLGA promoted higher reduction of the bacteria in comparison to the untreated samples. Confocal images of bacteria growth on different systems are reported in Figure 1 and confirmed the antimicrobial effect of treated films. Pristine films show a large number of viable bacteria (*E.coli* and *S.aureus*), (fluorescing green in Figure 1) on biomaterial surfaces, while after an exposure to oxygen plasma for 20min, a great number of dead cells (fluorescing red), are evidenced on treated PLGA and, more clear, on PLGA/7Ag treated surfaces.

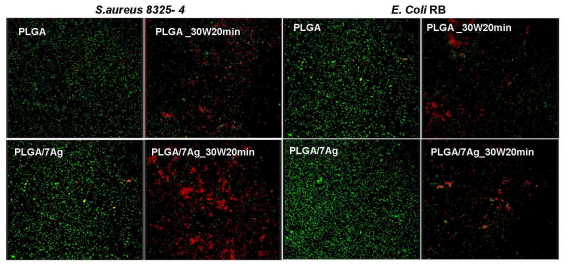

Figure 1: CLSM images of bacteria growth on materials indicated. Dead cells, which take up propidium iodide, fluorescence red, and cells fluorescing green are deemed viable.

Our observations clearly demonstrated that oxygen plasma surface treatment could readily improve the antimicrobial properties of nanocomposite PLGA/Ag surface.

Keywords biodegradable polymers; silver nanoparticles

Comparative study of the antibacterial activity of metal (silver, copper & zinc) doped sol gel coatings using microtiter wells

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The colonization of clinical and industrial surfaces with microorganisms, including antibacterial-resistant strains, has promoted increased research into the development of effective antibacterial and antifouling coatings. In the present study, the preparation of metal (Ag, Cu, Zn) doped methyltriethoxysilane (MTEOS) coatings and the rapid assessment of their antibacterial activity is described. The chemical interaction between the silane and metal salts were examined by ^{29}Si nuclear magnetic resonance (NMR). The wells of polystyrene flat bottom microtiter plates were coated using various sol gels and cured under controlled conditions. Curing parameters were analyzed by thermogravimetric analysis (TGA) and visual examination. The optimum curing range was determined when the wells were coated using various levels of sol gels. The coated wells were challenged with cultures of Gram-positive and Gram-negative bacteria, including antibiotic resistant organisms. Silver showed the highest antibacterial activity followed by zinc and copper. The silver doped sol gel had broad spectrum antibacterial activity making it potentially useful as a coating for biomaterials. The efficient use of microtiter plates enabled a variety of sol gel coatings to be screened simultaneously for their antibacterial activity against a wide range of bacteria.

Keywords: Antibacterial; Metals; Microtiter plate; Sol gel.

Coupling hydrodynamics and bio-chemical treatment: an alternative to improve biofilm removal

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Biofilm adhesion and growth occur in any system where microorganisms are present. Biofilms may be beneficial when used in bioreactors for water and wastewater treatment. Nevertheless, they can also affect profoundly both the human health and industrial productivity: they are responsible for infections and diseases and may also induce decreased efficiency, increased running costs, system damage or breakdown and deterioration in product quality in industrial systems. Furthermore, biofilms exhibit an increased resistance to antimicrobial agent leading to an important stake for researchers.

Biofilms can be defined as surface-associated layers of microbial cells embedded in extracellular polymeric substance (EPS). Thus several strategies have been developed to fight against such a complex matrix: avoid (or limit) their development and control their elimination. In this study we focus on the second one. To that purpose, literature report bio-chemical as well as mechanical treatments. Bio-chemical treatments consist in adding biochemical compounds to act against micro-organisms (antibiotics, (Abdi-Ali et al. 2006)), EPS (enzymes, (Xavier Joao B. 2005)) or both (oxidant agents, (Grobe et al. 2002)). Mechanical treatments use aggressive techniques that eliminate the biofilm deposited on the surface.

The increasing restrictive environmental regulations induce the necessity to take the environmental aspect into account in the choice of the suitable treatment. The use of substances that reduce reliance on inherently toxic antimicrobial agents, such as enzyme-based detergent also known as "green chemicals", would be an attractive strategy. However, using this kind of treatment in thick and mature biofilms, a limited penetration of agents is expected due to the low porosity inducing mass transport limitations. Therefore, there is a need to improve this treatment efficiency that could in turn decrease the environmental impact.

In this context, the aim of this study is to investigate alternative ways to improve biofilm removal, thus two complementary actions are studied and combined:

- hydrodynamic constraints to favor simultaneously surface erosion (a cohesive basal layer, that can't be removed by hydrodynamics, has been however put in light (Coufort et al. 2007)) and mass transport.

- Enzyme injections to act on the physical integrity of the EPS matrix to reduce the basal layer cohesiveness.

To test this combined treatment, biofilms are developed on plates under constant low shear stress until steady-state is reached. Then they are subjected (1) to erosion tests performed on systems with different hydrodynamic conditions or (2) to bio-chemical tests or (3) to erosion tests followed by bio-chemical tests.

Results will be analyzed based on characterizations of biofilm (thickness, DCO_X, biofilm mass) performed prior and after each treatment. Those physical properties will be compared to the decrease of activities evaluated by respirometric measures (at macroscale with respirometric assays and microscale with microelectrodes).

Main results are that hydrodynamic action allows removing around 80% of the biofilm. The 100µm thickness basal layer is then more accessible to enzyme attack. Experiments using this combined treatment are currently undergone in paper industry.

Keywords: biofilm; biofouling; enzymes; cohesiveness; hydrodynamics; mass transport; detachment.

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Cronobacter biofilms; the effect of growth media and temperature on formation, and resistance to various environmental stresses

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Cronobacter spp. are regarded as emerging opportunistic food-borne pathogens causing rare outbreaks of enteritis, septicaemia and meningitis in neonates and infants. The vehicle implicated for their dissemination in most reported outbreaks was powdered infant formula (PIF). Despite the rarity of cases, the severity of illness (including high case mortality) has raised the concern of healthcare workers, regulatory agencies and manufacturers of PIF. We previously conducted a phenotypic and genotypic study characterising a collection of 64 *Cronobacter* isolates with most (53 isolates) being identified as *C. sakazakii* (remaining isolates included five *C. malonaticus*, three *C. muytjensii*, two *C. dublinensis* and one *C. turicensis*) (1).

In the present study we examined the biofilm forming capacities of the same isolates on abiotic surfaces. Biofilm formation was initially assessed by the crystal violet (CV) microtitre assay in polystyrene plates using four different broth culture media (AB minimal growth medium (AB), Luria Bertani (LB), brain heart infusion (BHI), and 1/10 strength commercial infant formula (IF)). CV staining was carried out after 48 h growth at either 22°C or 37°C. Biofilms were quantified by absorbance measurements at 570 nm and isolates were grouped into 4 categories (no biofilm, weak, moderate, or strong). Biofilm formation was dependent on growth medium and to a lesser extent, temperature. For example, when grown in BHI most isolates produced weak biofilms or were non adherent (only two isolates produced moderate to strong biofilms). Similarly, 85% of isolates grown in LB were non- or weak biofilm producers. Conversely, when either AB or IF were used as the growth substrates, moderate to strong biofilms were observed for the majority of isolates: AB, 67% and 55% and IF, 75% and 69% at 22°C and 37°C, respectively. We also examined biofilm formation on food grade stainless steel (SS) using the CV microtitre plate assay. Type 304, no. 4 finish SS coupons (0.7 cm diameter) were wedged into microtitre plates wells and the assay was conducted using three media (AB, BHI, 1/10 IF) at 25°C. After 48 h, coupons were removed, rinsed with PBS and stained with CV. Under these test conditions 75% of isolates displayed moderate to strong affinity for SS when grown in either BHI or IF, and 69% showed similar affinity when grown in AB. Scanning electron microscopy (SEM) of SS coupons revealed thick multilayered congregations of cells embedded in a matrix, especially at the surface interface.

Further experiments were conducted to compare the resistance of biofilms with planktonic cells for 4 isolates; HMPA02 (*C. turincensis*), HMPA 87A (*C. malonaticus*), CHPL 02 (*C. sakazakii*), and CHPL67 (*C. muytjensii*); to various stress factors (sodium hypochlorite, 200 mg/L; high acidity, pH 3.0; H_2O_2, 1g/L; ethanol, 20% (v/v); and high osmolarity, NaCl, 30% (w/v)). Static biofilms were prepared on glass cover slips using 1/10 IF as the growth medium then exposed to each stress treatment for prescribed time periods prior to enumeration. Resistance to each stressor varied with strain and cellular state. For HMPA02 significantly more survivors ($P < 0.05$) were observed for biofilm cells in all treatments. However, this trend was not universal as biofilms of the other tested strains did not display the same level of resistance for all treatments. Although biofilms of CHPL02 and CHPL67 were more resistant to ethanol, high acidity and H_2O_2, planktonic cells for both strains appeared to be equally resistant to sodium hypochlorite. Moreover, the biofilm cells for CHPL67 exhibited increased sensitivity to high osmolarity.

This study contributes to the growing body of literature on the ecology and physiology of *Cronobacter* spp. Understanding the conditions leading to biofilm formation by these pathogens will enable the design of effective strategies to eliminate or minimize their persistence in facilities where PIF is processed.

Key words: *Cronobacter* spp., biofilms, stress resistance

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Cyclic AMP regulation of *Vibrio cholerae* virulence and development of a cell-based assay for agonists and antagonists of the bacterial cyclic AMP receptor protein

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Cholera is an acute water-borne diarrheal disease caused by *Vibrio cholerae* of serogroups O1 and O139. The hallmark of choleragenic *Vibrios* that cause epidemic cholera is the expression of two major virulence factors: the toxin co-regulated pilus (TCP) required for intestinal colonization and cholera toxin (CT) responsible for the profuse rice-watery diarrhea. The cAMP receptor protein (CRP) has been reported to enhance virulence gene expression in several pathogenic members of the *Enterobacteriaceae* and *Vibrionaceae* families. To better understand the role of CRP in cholera pathogenesis we have constructed isogenic Δ*crp* and Δ*cya* mutants, a strain expressing a constitutive *crp* allele ($crp^{T127L/S128A}$) active in the absence of cAMP as well as a $crp^{T127L/S128A}$Δ*cya* double mutant. In spite of expressing more CT and TCP *in vitro*, Δ*crp* mutants are defective in intestinal colonization. We show by global gene expression profiling that Δ*crp* mutants express lower levels of multiple genes encoding determinants of motility, chemotaxis, outer membrane proteins and stress response required to establish infection. On the other hand, a strain expressing a constitutive (cAMP-independent) CRP expressed lower TCP, CT and was also defective for intestinal colonization. These results suggest that *V. cholerae* needs to regulate the activity of CRP during infection to cause disease and that both lack of CRP and permanent activation blocks the infective process. Consequently, assays for CRP agonist and antagonists could potentially identify lead compounds with therapeutic and prophylactic potential, respectively. Here we also present a simple and robust assay for agonist and antagonists of CRP based on our finding that this protein controls the expression of the master quorum sensing regulator HapR that can activate the *V. harveyi lux* operon. Briefly, Δ*cya* mutants containing the *lux* operon are dark and light production can be restored by exogenous cAMP or its analog 7-deaza-cAMP. On the other hand, light production from wild type *V. cholerae* containing the *lux* genes could be quenched by cGMP which binds the CRP protein but does not induce the correct allosteric change required for this regulator to activate transcription. Data is presented for the validation of these assays using a 384-well plate format. This work was supported by funding from Southern Research Institute and PHS Grant AI63187 to J.A.B.

Keywords Cyclic AMP; Cyclic AMP receptor protein; quorum sensing; *Vibrio cholerae*; high throughput screening

Determining of Effectiveness of Organosulfur Compound with Using ATP Luminometer in the Leather Industry

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Organosulfur compound is the world's leading bactericide product for the leather industry. It was reported that it's highly effective for preserving salt and brine solutions, soaking liquors as well as processing materials[1].

The objective of this study was to examine the effectiveness of organosulfur compound commercially with using ATP Luminometer. According to ATP test, organosulfur compound has enough effective bactericidal activity during latent period in soaking process.

Keywords: Leather industry, Soaking, Organosulfur compound, ATP.

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Development of Broad Spectrum Anti-Microbial Surfaces Containing a Variety of Amphiphilic Active Compounds

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With the increase of antibiotic resistant microbes, the production of self-decontaminating surfaces has become an area of research that has seen a surge of interest in recent years. Such surfaces, when incorporated into commercial products such as children's toys, medical devices and hospital surfaces could reduce the number of infections caused by pathogenic bacteria. A number of active components for self-decontaminating surfaces have been investigated, including common antibiotics, silver ions, quaternary ammonium salts (QASs), and anti-microbial peptides (AMPs). A recent research focus has been development of a wide range of amphiphilic anti-microbial additives that when combined with modern low volatile organic compound (VOC), water based paints leads to a surface concentration of the active compounds as the coating cures. Herein we report the development of amphiphilic QASs and AMPs with the ability to surface segregate within a coating. These coatings have demonstrated the ability to reduce by 1-7 logs potential pathogens which come into contact with the surface, including various bacteria, viruses, and fungi.

Keywords: quaternary ammonium salt, peptide, coating

Effect of silver nanoparticles on the bacteria adhesion, antibacterial activity and silver ion release of biodegradable polymer nanocomposite

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In the past several decades nanoparticles have been the focus of intense researches not only due to their novel properties, which differ greatly from the bulk materials, but also for their wide practical applications. Silver (Ag) nanoparticles have found wide applications in catalysis, antimicrobials, conductive inks and electronic devices. Therefore, functional nanocomposites with desired properties can be tailored by incorporating Ag nanoparticles into the polymers. Silver-ion releasing compounds and in particular silver nanoparticles, have drawn considerable interest for their capability to release silver ions in a controlled manner which in turn leads to a powerful antibacterial activity against a wide spectrum of bacteria.

In this work, silver nanoparticles have been prepared by use of selective reduction of Ag+ ions by citrate in the presence of polymeric stabilizers to control and orient the particle growth. Synthesized silver nanoparticles (n-Ag) with a diameter ranged between 4÷40nm were obtained. Biodegradable nanocomposites based on poly(DL-Lactide-co-Glycolide) (PLGA) copolymer and certain amount of n-Ag were produced by means solvent casting technique.

PLGA nanocomposites based on commercial silver nanoparticles (Ag) were also produced and analyzed.

Field Emission Scanning Electron Microscopy (FESEM) and Confocal Laser Scanning Microscopy (CLSM) investigation were performed to evaluate the nanocomposite morphology and nanoparticle dispersion. Surface properties of the films were investigated by Atomic Force Microscopy (AFM) and Contact Angle (CA) measurements. Antibacterial tests were performed by measuring the growth of E.coli in LB broth medium in the presence of nanocomposites and also the bacterial adhesion capacity on the nanocomposite surfaces was tested. Silver ion release was also evaluated for both commercial and synthesized nanoparticles embedded in the polymer matrix.

Morphological observation of PLGA nanocomposite upper surface underlined the presence of a superficial and circular porous structure with a pore diameter of about 10μm on PLGA/commercial Ag nanocomposite while a not regular roughness was found in the case of synthesized particles.

The adhesion tests conducted on PLGA nanocomposite loaded with commercial silver revealed that the efficiency of *E. coli* to adhere on nanocomposite surfaces is strongly reduced compared to the PLGA film and the bacterial growth was almost inhibited on the rough surface of the nanocomposites compare to the neat polymer.

The Ag+ release was monitored through periodic analysis with ICP technique. The release of Ag+ for a given commercial silver content is characterized by a sigmoid trend. Moreover, the release is relatively slow at the beginning until 20 days of incubation and becomes faster with the incubation time until 70 days in aqueous solution. Starting from 80 days of incubation a saturation limit is reached.

A completely different behaviour was detected for synthesized particles: ions release already begun 24h after the exposition to the degrading environment. Nanocomposite with n-Ag follow an exponential trend and after an initial intense release phase, the materials quickly reached a plateau.

This analysis underlined that nanocomposites based on synthesized Ag were more reactive towards the external environment respect to the commercial ones.

These studies suggest that PLGA properties can be modified introducing a small percentage of silver nanoparticles. By processing biopolymers with metal nanoparticles, it is possible to change matrix properties and to develop hybrid enhanced materials. The combination of biodegradable polymers and silver nanoparticles opens a new perspective in the self-assembly of nanomaterials with tune-able thermal and morphological properties and thereafter it is possible to obtain antibacterial surface for biomedical application.

Keywords silver nanoparticles; silver ion release

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Effect of sub-MIC concentrations of chitosan on *Porphyromonas gingivalis* biofilm formation

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Oral biofilms are fundamental in the ethiology of oral diseases such as periodontitis in which *P. gingivalis*, a Gram-negative anaerobic and asaccharolytic bacteria is generally recognized as predominant. Nowadays, new approaches to combat oral biofilms based on biomaterials are being pursued. In that light, chitosan a product of heterogeneous alkaline *N*-deacetylation of chitin, with strong antibacterial activity is one of most promising. In this study we used sub-MIC concentrations of high and low molecular weight chitosan (HMW; LMW), in medium supplemented with 5% sucrose, to determine the capacity of chitosan to inhibit biofilm formation. The results showed that sub-MIC concentrations of both chitosans were very effective in inhibiting biofilm formation at 0.05% (v/v). HMW chitosan presented an inhibition of 88.23% and LMW chitosan 86.35% after a week. In addition, the presence of chitosan in the media lowered initial pH levels, which further contributed to the high efficacy of biofilm inhibition.

Keywords: *P. gingivalis*, biofilm formation, sub-MIC, chitosan

Efficacy of nisin-activated plastic films against meat spoilage bacteria *in vitro* and in meat

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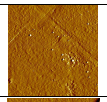
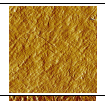
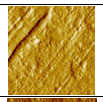
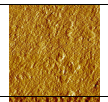
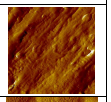
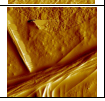
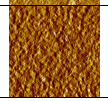
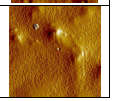
In this study, different commercial polyethylene (PE) films differing in ethylene vinyl acetate, erucamide contents and oxygen permeability were employed for the development of active packagings. A nisin-based solution was used for the activation of the plastic films; the activity of the antimicrobial solution and of the packaging materials after activation were assayed using different spoilage associated bacteria as indicator strains. The antimicrobial solution was spread manually on food contact layer of the different plastic films using coating rods providing nominal thickness of 6, 40, 60 and 100 µm. The polyethylene films before and after treatment were analysed by Atomic Force Microscopy (AFM) in order to have information on the distribution of the bacteriocin solution on the plastic layer. The antimicrobial solution was active against Gram-positive bacteria and the highest activity in terms of AU/ml was obtained against *Brochothrix thermosphacta*. The AFM images of surfaces of control and activated plastic films are shown in Figure 1. The most homogeneous spreading of the NS onto the films surface, as determined by AFM analysis, was obtained by the coating procedure with 100 µm of thickness on the E0E0P074 and HDPE films. The same films were active against all the Gram-positive bacteria. The AFM analysis followed by the calculation of roughness parameters showed that the plastic films E5E600P1 and E0E0P074 treated with the antimicrobial solution had the highest roughness values than all the analyzed films. Viable staining and epi-fluorescence microscopy analysis of culture suspensions of the indicators strains in direct contact with treated and control films proved that the different indicator strains had different sensitivity according to the PE film used in terms of damage and % of viability reduction. Therefore, the antimicrobial efficacy of the films was dependent not only on the strain but also on the type of film activated.

Furthermore, beef chuck tender slices were purchased from butcher shops and two portions of 5 cm diameter were aseptically cut from each beef slice and covered with the antimicrobial plastic films on both sides. After 1 h and 1, 7, and 12 days of storage at 4°C the meat samples were analyzed by viable plate counts targeting the following microbial populations: lactic acid bacteria (LAB), *Enterobacteriaceae*, *Pseudomonas* spp. and *Brochothrix thermosphacta*. The antimicrobial films after 1 h of contact with the meat caused a significant reduction of 0.5 and 1 log cycles of LAB and *Brochothrix thermosphacta,* respectively. The most effective antimicrobial activity of films was shown against the same populations after 24 h of storage. In conclusion, antimicrobial packaging can be considered an extremely challenging technology that could have a significant impact on shelf-life extension and food safety of fresh meat and meat products.

This study was partly supported by a EU project (SYMBIOSIS-EU) within the 7th Framework Programme (ref. Grant agreement N°. 211638).

Keywords nisin-activated antimicrobial packaging; meat spoilage

Figure 1 - Representative images from AFM analysis (20 x 20 µm) of different types of polyethylene films treated or not with the antimicrobial solution.

Plastic film	0E0P074	E0E350P89	E0E500P9	E0E450P9	HDPE
Control film					
Treated film					

Efficacy of water medication with enrofloxacin in turkey colibacillosis: preliminary data.

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The widespread use of antimicrobial agents in food animals can be linked to an increase in antimicrobial resistance, also in food-borne pathogens, which may subsequently be transferred to humans. The use of fluoroquinolones in poultry is largely diffused in Europe, whereby Enrofloxacin (ENR), developed exclusively for veterinary use, is the most commonly used. A PK/PD model was applied to confirm the efficacy of authorised water medication with ENR in turkeys.

Sixty field strains of avian pathogenic *E. coli* were isolated from commercial poultry affected by the clinical disease. The isolates were tested for ENR sensitivity by both the Agar Disc Diffusion Method (Kirby-Bauer, 1966) and the MIC Microdilution Method (NCCLS manual M31-A2). The results showed a very good correlation for the discrimination between sensitive and resistant strains. Thus the Agar Disc Diffusion Test, frequently used in the field for diagnosis, has been confirmed as an effective and economic screening method to select antibiotic therapy.

Kinetics of ENR and its active metabolite Ciprofloxacin (CIP) were then evaluated in twenty turkeys: 10 healthy, and 10 infected with *E. coli* and severely ill. All turkeys were administered ENR *per os* at 10 mg/kg for 5 days *via* medicated water, using a commercial product.

Blood samples were collected from the healthy turkeys for 24 h on days 1 and 5 of treatment and serum was analyzed for ENR and CIP concentrations with High Performance Liquid Chromatography (HPLC) with fluorimetric detection. No cumulating effects emerged during this treatment. Blood samples were collected from the ill turkeys for 24 h only on day 5 to reduce stressful procedures, and subsequently analysed. With oral administration for 10 hours, as used in the field, the kinetic curves of the healthy and ill turkeys are similar and both remarkably different from literature data. The therapy does not match PK/PD break-point also when sensitive strains (MIC values 0,25 and 0,125) are considered.

The main kinetic parameters from the three groups and MIC values were compared and results discussed considering the need for harmonising veterinary drugs containing quinolones or fluoroquinolones authorised for all food-producing species in Europe, as stated by EMEA/CVMP/268320/2009.

Keywords *E. coli*; turkey

Engineering of non-adhesive glycocalyx-like nanofilm on silicone surfaces using a "clickable" methylcellulose

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The uncontrolled accumulation of biological material on the surface of devices placed in contact with biological media fails to replicate the natural structure and function at the body site and typically causes adverse biological reactions in the living host system. Silicone elastomers (polydimethylsiloxane (PDMS)) have many attributes that make them excellent materials for biomedical applications but their hydrophobicity is particularly prompt to generate a strong bioadhesion. Here, we report the design of new active polysaccharide nanoassembled surfaces that by mimicking the non adhesive properties of the glycocalyx allow the bioadhesion control. These nanoassemblies consist of a methylcellulose layer grafted in one single step, in water, on unmodified commercial PDMS surface via an orthogonal click reaction. The resulting biomimetic surface is effective in suppressing protein adsorption, bacterial and mammalian adhesion. This first example of a new class of biologically inspired surfaces should have great potential in the design of various devices aimed to easily trigger and modulate the bioadhesion in the field of implantable biomedical devices as well as microfluidic devices.

Keywords polydimethylsiloxane; PDMS; surface; antiadhesive

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Evaluation of fungi grow using essential oils from garlic and onion after ionizing process

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Brazil is the largest pesticides consumer in Latin America, using about 1.5 kg of active ingredients per hectare of crop. New alternative methods of pest control aimed to reduce the use of fungicides from raw materials of botanical origin have been investigated in several cultivars. The essential oils have been shown to be potentially useful.

The citrus species are primary hosts a lot of pests of polyphagous feeding habits that can infest more than 300 plant species, including among them, mango, grapes, cashew, avocado, guava, apple, fig, banana, papaya, pear, pomegranate, quince, coffee, roses and other. Direct damage is caused because of continuous sucking nutrients from leaves and the consequent depletion of the plants by nymphs and adults. The indirect damages are caused by the elimination of sugary secretion on the leaves, inducing the occurrence of saprophytic fungi (fumagina) which forms a pellicle with an aspect of sooty, that reduce considerably the incidence of light consequently decreasing the photosynthesis. Considering the inhibitory property of essential plant oils on the mycelial development of fungi, as well as the economic importance of fumagina in citrus, this research aims evaluate the toxicity of essential oils (extracted from garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) after the radiation treatment normally utilized as an inhibitor of growing for these two vegetables), in fungi belonging to the group *Capnodium ssp* isolated from citrus leaves. The ionizing process will be done (Carried out) in a ^{60}Co source Gammacell 220 (A.E.C. Ltda) at doses of 0, 1.0 and 2.0KGy.

Keywords: Food irradiation, Garlic, Onion, fungi, essential oil, inhibition

Exploring thermostable quorum quenching lactonases to counteract bacterial infections in cystic fibrosis

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Pseudomonas aeruginosa is the major cause of clinically relevant infections in cystic fibrosis (CF) due to antibiotic resistance, which demands for new strategies of microbe proliferation control. CF represents a model biofilm infection. During the course of CF, P. aeruginosa starts to produce exo-polysaccharides and forms biofilms characterized by cellular aggregates embedded within the mucus layer present in the airways. ofilm formation in the CF lung The question of whether there is direct involvement of the QS system in biofilm formation has been a key issue for some time. However, in a mouse infection model, QS inhibitory drugs reduce biofilm formation. In addition, human PON1 inhibits P. aeruginosa biofilm growth in an in vitro biofilm model as well as in an in vivo animal model. QS is an interbacterial mode of communication accomplished through the coordinated production, secretion, and detection of chemical signals (QS signals) that trigger the expression of specific bacterial genes. The QS signals self-produced by P. aeruginosa are in the form of small molecules, or autoinducers, termed acyl-homoserine lactones (acyl-HSL). P. aeruginosa uses acyl-HSL quorum-sensing molecules to regulate the expression of genes implicated in virulence and biofilms formation. Promising new therapies that target biofilm formation include molecules (quenching enzymes or small molecules) that disrupt QS. It has been shown that all of the human PONs can inactivate 3OC12-HSL .

These observations indicate that quorum-sensing molecules are important for *P. aeruginosa* virulenceand biofilm formation and that lactonases, such as PONs, can protect the host from lethal *P. aeruginosa* infection. We propose to exploit recently characterised phosphotriesterase-like lactonases (PLLs) from thermophilic microorganisms to counteract *Pseudomonas* infection in the CF patients. We will report on the main properties of these PTE-like lactonases. These enzymes are characterised by high resistance to several stressing factors other than temperature. The effect on Sulfolobus solfataricus as well as Pseudomonas aeruginosa PO1 growth will be reported and discussed.

Keywords:quorum quencing, thermostable lactonases

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Fabrication and enzymatic biofunctionalization of nanofibers as a new material for wound dressings

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In past years nanomaterials have become one of the most popular scientific area not only in technical field but also in biomedicine. Goal of our work was to fabricate cellulose and chitosan nanofibers and to biofunctionalize them by lysozyme and trypsin. Nanofibers were prepared by technology Nanospider^{TM}, a unique technology based on electrospinning process that enables production of nanofibrous textiles on an industrial scale. Cellulose nanofibers (Fig 1) were obtained by electrospinning of cellulose acetate and the nanofibrous layer was afterwards deacetylated. Chitosan nanofibers were prepared by electrospinning of acidic chitosan solution and the nanofibrous layer was subsequently crosslinked by heating. The fabrication parameters (electrode distance, voltage, electrode rotation, climatic effects, etc.) were optimized for obtaining desired weight area and diameter of nanofibers. For both enzymes the bond formation and enzyme stability was firstly pretested on model system with magnetic cellulose microparticles (MPs). The lysozyme-MPs bioreactor was successfully used for cell wall fragmentation of *Staphylococcus aureus* and *Bacillus subtilis*. After that the enzymes (lysozyme, trypsin) were covalently immobilized on the surface of nanofibers and the enzymatic activity was measured. The effect of immobilization procedure and reaction conditions on enzyme activity as well as storage and operational stability was observed. Finally, inhibition tests (influence of pH, ionic strength, effect of desiccation, etc.) important for practical use of the newly developed material were performed. We tend to focus on suitable non toxic or irritant solvents, crosslinking agents and substrate materials since the material has a strong potential for utilization in biomedicine. Our first results indicate that biofunctionalized nanofibers could be a new promising approach for wound treatment.

The authors wish to acknowledge the Ministry of Education No. MSMT 0021627502 and Ministry of Industry and Trade No. MPO TIP FR-TI1/0436 for financial support of research program.

Keywords: nanofibers; biofunctionalization; enzymes

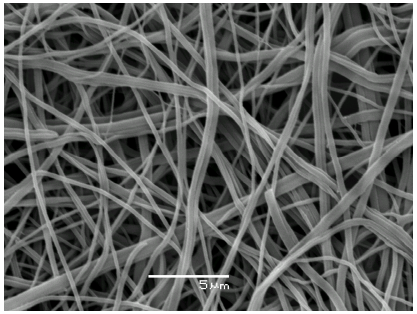

Figure 1 SEM image of cellulose nanofibers prepared by electrospinning technology. Coated with a layer of gold (Jeol JSM 5500 LV SEM).

Factors affecting adhesion of *Staphylococcus aureus* in different surface.

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Depending on the demand for healthier foods and convenience, the researchers acknowledge that there is a growing need for mathematical models that describe the behavior of micro-organisms under different conditions. This study aimed to use the surface response methodology (SRM) to assess the effect of different combinations of temperature, pH, NaCl concentration, concentration of NaNO2 and contact time, applied simultaneously, in the adhesion of *Staphylococcus aureus* on surfaces of stainless steel (STS), polyurethane (PU) and polyvinyl chloride (PVC). The experiments were conducted in accordance with the Box-Behnken design, being therefore, selected three levels of each independent variable studied. The lower, central and upper levels of each variable were designated as -1, 0 and +1. 40 combinations between the levels of five factors and six replicates of interest regarding the combination at the center point to estimate the experimental variance were tested. The Minitab® 14 program was used in the statistical analysis. *S. aureus* was able to adhere, under certain conditions, on the surfaces of STS, PU and PVC. On all studied surfaces the extreme levels of the factors provided lower adhesion ability of the microorganism to the surface, being the best condition of the adhesion close to the studied central point. It was observed that the adherence of *S. aureus* to the surfaces becomes more pronounced as the contact time increases. None of the NaNO2 concentrations used in this study were significant in the adhesion of *S. aureus*. The results indicated that the conditions used in the manufacture of fermented meat products such as low pH achieved in a short period of time and the packaging of the product at low temperatures, besides decreasing the proliferation of *S. aureus* in this product may prevent the micro-organism to start the adhesion process on the surfaces of STS, PVC and PU. The high coefficients values of determination (R^2) demonstrated the suitability of the quadratic model to explain the influence of independent variables in the response. On the surface of STS the model accounted for 77.5%, 74.8% in PU and in PVC 75%. The bias factors (bf) calculated for the surfaces of STS, PU and PVC were 1.05, 1.05 and 1.08 respectively, while the accuracy factors (af) were 1.18, 1.16 and 1.82 respectively . The models generated in this study were classified as good and acceptable and thus can be used to predict the adhesion of *S. aureus* on the surfaces of STS, PU and PVC.

Keywords: Adhesion, *Staphylococcus aureus* and predictive microbiology.

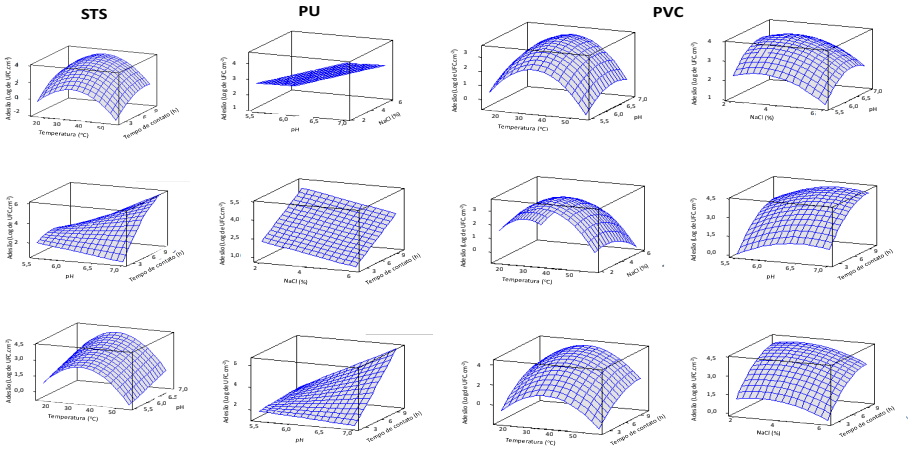

Figure: Response surface plot of adhered cell count of S. aureus in different surfaces.

Formation of Curcumin Nanoparticles using Rapid Expansion of Subcritical Solutions into Liquid Solvents

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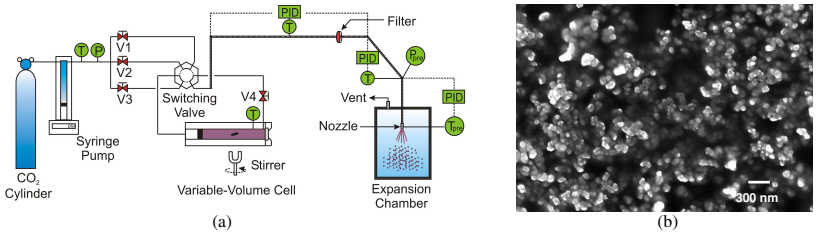
Curcumin is a major active compound of the tropical herb *Curcuma longa* with antimicrobial effect against many microorganisms, especially *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*. However, its poor water solubility limits the use in food processing industry. This could be achieved by decreasing the particle size in order to increase the surface area and improve the dissolution rate of the active component. Conventional techniques for producing nanoparticles still have several limitations, including generating broad particle size distributions and requiring large amounts of surfactants. Rapid expansion of supercritical solutions into liquid solvents (RESOLV) can produce organic nanoparticles. When a supercritical solution containing a solute and a solvent (typically carbon dioxide, CO_2) is rapidly expanded across a nozzle, the solvent density suddenly decreases, leading to precipitation of the solute in the form of fine particles. Most rapid expansion studies have focused only on rapid expansion of the solutions in the supercritical state. Therefore, it remains challenging to produce nanoparticles through rapid expansion of subcritical solutions at conditions below the critical point. The objectives of this work were to determine the feasibility of rapid expansion of subcritical solutions to produce curcumin nanoparticles and to investigate the effect of pre-expansion temperature (T_{pre}) on the size and morphology of curcumin particles. The obtained products were characterized using Field Emission Scanning Electron Microscopy (FESEM), UV-Vis spectrophotometry, and Fourier Transform Infrared (FTIR) spectroscopy. For rapid expansion experiments, the subcritical solution contained a solvent mixture (ethanol+CO_2 1:1 wt/wt) and curcumin (0.1 wt%) at a constant pre-expansion pressure (P_{pre}) of 330 bar (see Fig. 1a). It was found that spherical curcumin nanoparticles with a size range of 30–80 nm and average sizes of ~40–50 nm were successfully produced by rapid expansion of subcritical solutions into an aqueous receiving solution containing 0.1 wt% Pluronic F127 (stabilizing agent) (Fig. 1b). In addition, an increase of T_{pre} from 50 to 80°C increased the extent of particle agglomeration without significantly affecting the particle sizes. Investigations of antimicrobial activity of curcumin nanoparticles are currently underway.



(a) (b)

Fig. 1 (a) Schematic of rapid expansion of subcritical solutions into liquid solvents apparatus and (b) FESEM micrograph of curcumin nanoparticles produced by the rapid expansion process.

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Keywords curcumin; nanoparticles; antimicrobial activity; carbon dioxide; subcritical solutions; rapid expansion

Functionalized Microparticles with Biocide Properties

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Biofilms are ubiquitous in nature and its formation is a strategy that bacteria use in order to survive in hostile environments. Although biofilms play crucial roles in many processes (like the biodegradation of environmental pollutants or the microbial balances within a body) they often are unwanted and cause serious problems in various areas like the food industry, medical industry, industrial heat exchangers and cooling water systems, which can lead to increase in the costs of production and maintenance, as well as to public health concerns and environmental impacts. This promoted the use of disinfectants and antiseptics. To combat the growth and proliferation of these biofilm, chemical products with antimicrobial properties like biocides and surfactants are being used, but these compounds are obviously deleterious to the environment and consequently should be use in as small quantities as possible.

Nano-micro-technology can present a unique alternative as detection methods of bacterial targets. Nano- and microparticles present other advantages like high reactivity, unique interactions with biological systems, small size and large surface to volume ratio optimized for mass loading and carrying of antimicrobials like biocides. Antimicrobials can be loaded into nano-micro-particles by physical encapsulation, adsorption or chemical conjugation and this can present several advantages such as significantly improve the activity of the antimicrobial, in contrast to the free product, and the release of the antimicrobial at a sustained and controlled manner. The Layer-by-Layer (LbL) self-assembly of oppositely charged polyelectrolytes onto colloidal particles has been used to create novel microparticles with well controlled size and shape, finely tuned wall thickness and variable wall compositions. The original method was introduced in 1991 by Decher and co-workers for the construction of pure polymer multilayer films on planar supports (Caruso 2001).

The efficacy of the method was assessed against biofilm of *Pseudomonas fluorescens*, using benzyldimethyldodecylammonium chloride (BDMDAC), a quaternary ammonium compound belonging to the family of benzalkonium chloride, as a biocide carried on polystyrene microparticles (diameter: 4 µm). The BDMDAC functionalized microparticles were prepared using the layer-by-layer self-assembly (LBL) technique. The oppositely charged polyethyleneimine, sodium polystyrene sulfonate and BDMDAC were assembled on polystyrene cores. *P. fluorescens* biofilms were developed on polyvinyl chloride surfaces in a chemostat for 7 days (Ferreira et al. 2010). The biofilm control activity of functionalized microparticles with BDMDAC was carried out through the determination of the survival ratio of *P. fluorescens* biofilm cells after different exposure periods to BDMDAC coated particles. For comparative purposes, the biofilm control effects of free BDMDAC were also tested against *P. fluorescens* biofilms. The biofilm exposure to these particles (0.87 mg/L) resulted in a viability decrease of 60.5% and 66.5% of the total biofilm population for 30 and 60 min exposure time, respectively. The exposure during 60 min to 6.33 mg/L and 11.75 mg/L of BDMDAC in particles with antimicrobial promotes inactivation of 80.6 % and 87.2 % of the total population, respectively.

The overall results indicate that this novel antimicrobial strategy has potential application on the control of microbial growth within biofouling. Moreover, this technique allows the reuse of the antimicrobial molecules, minimizing environmental risks associated with abusive use of antimicrobial agents with real benefits to public health. After 18 months in borate buffer pH 9 (kept at 4 ºC), the particles coated with BDMDAC released only 15% of the QAC. The possibility of reusing the BDMDAC coated microparticles to increase their life time and save biocide was also studied in order to optimize the industrial cleaning procedures.

At this moment, we are testing calcium carbonate microparticles (diameter: 3 µm), that present the advantage of being cheaper and highly abundant with preliminary experiments showing promising results comparatively to the polystyrene microparticles carrying the antimicrobial.

Keywords: microparticles; biofilms; biocides; drug-delivery; *Pseudomonas fluorescens*

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Ferreira, C., R. Rosmaninho, M. Simões, M. C. Pereira, M. M. S. M. Bastos, O. C. Nunes, M. Coelho and L. F. Melo "Biofouling control using microparticles carrying a biocide." Biofouling: The Journal of Bioadhesion and Biofilm Research 26(2): 205 - 212 (2010).

Furanones and thiophenones in controle of *S. epidermidis* biofilm infections?

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Coagulase-negative staphylococci (CNS) are commensal bacteria found on human skin and mucous membranes. The CSN *Staphylococcus epidermidis* was long considered harmless. In the past decade it has emerged as one of the most frequent CNS isolate, as well as a major nosocomial pathogen often found in implant associated infections related to various catheters or orthopaedic devices. The main virulence factor of *S. epidermidis* is related primarily to its ability to form biofilms on surfaces of medical devices. There are few available means to effectively prevent or treat medical device-related infections. *S. epidermidis* biofilm infections have thus become an increasing burden to the public health system, and in many cases being responsible for prolonged hospitalisation and mortality. Since infections associated with biofilms are difficult to treat due to enhanced resistance both to antibiotics and the human immune system, there is great interest in finding alternative approaches to prevent and to treat biofilm related infections. So far, antimicrobial coating of implants is the most frequently used method to prevent *S. epidermidis* infections. However, the effect seems to be of short duration and mightlead to antibiotic resistance development.

We have synthesised furanones similar to those found in *Delisea pulchra* and have shown that they may interfere with bacterial communication. Therefore furanones and similar compounds may represent promissing alternatives for preventing biofilm related bacterial infections.

AIMS: The aim of this study was primarily to assess wether a thiophenone compound with structure similar to a previously tested furanone, would be as effective in preventing biofilm formation.. A second aim was to investigate wether the effect was a result of interference with bacterial communication, and finally to compare the thiophenone and the furanone for their possible irritative effects..

METHODS:First the thiophenone and the furanone were tested in a biofilm microtiter-based assay. The two agents were then assessed for ability to interfere with bacterial communication by the *V. harveyi* bioluminescence method. Finally irritability upon exposure to sensitive tissue was assessed according to the HET-CAM (Hens Egg Test-Chorioallantoic Membrane) protocol

RESULTS: Biofilm formation by *S. epidermidis* was significantly reduced by the thiophenone tested. The thiophenone was more effective in preventing biofilm formationthan the furanone. In the communication assay, bioluminescence was reduced by the thiophenone to a greater extent than with the furanone. The thiophenone was found to be non-irritative at the concentrations used, but an effect on sensitive tissue was observed at lower concentrations than what was observed with the furanone.

Conclusion: Thiophenones thus represent promising agents for protecting surfaces from being colonized by *S. epidermidis*.

Keywords keyword; keyword

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Gamma radiation effects on bacteria and fungi in coffee (*Coffea arabica* L.)

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Microbiological contamination of coffee (*Coffea arabica L.)* is a problem in the industry, as coffee is especially sensitive to contamination. Coffee is susceptible to bacteria and fungi contamination at all plant stages, from production to consumption. The occurrence of fungi and bacteria causes modification in the color, and flavor, also loss of weight and the development of secondary metabolites that are toxic to humans. Irradiation is a decontamination method use for a variety of foodstuffs, being very feasible, effective and environment friendly one without any significant change of sensory properties of the coffee. The aim of this study was to verify the effect of different gamma irradiation doses (0; 1, 5 and 3kGy to control bacteria and 0; 3; 6 and 9kGy to control fungi) on roast coffee grains to determine if the microbiological load which is often present in coffee was destroyed. The analysis were performed to determine the fungi contamination and *Salmonella ssp* the results were expressed as the viable counts per gram of sample (CFU/g), and coliforms presence in roast coffee samples irradiated and unirradiated. The results shows that microbiological contamination of coffee decrease when increase radiation doses.

Keywords: coffee; irradiation; fungi; *Salmonella ssp*; coliforms

Harnessing the antibacterial potential of silver nanoparticles against burn and wound pathogens

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Silver nanoparticles are found to possess remarkable antibacterial property against a wide range of microorganisms. The present study incorporates our report on phytofabrication of silver nanoparticles using *Thevetia peruviana* and its *in vitro* antibacterial activity against burn and wound pathogens. The bactericidal activity of silver nanoparticles was evaluated by disc diffusion, MIC and MBC methods. Phytofabricated silver nanoparticles showed potent antibacterial activity against *Escherichia coli*, *Streptococcus pyogenes* and *Acinetobacter bauminii*. The silver nanoparticles showed maximum inhibition against *Acinetobacter bauminii* while, minimum inhibition was observed against *Staphylococcus aureus*.

Keywords: Nanotechnology, nanomaterials, antimicrobial, Phytofabrication, Silver nanoparticles, *Thevetia peruviana*, TEM.

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Human antimicrobial peptide LL37 inhibits biofilm formation of *Staphylococcus epidermidis* on polyurethane central venous catheter

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Biofilm-associated catheter infection is a common complication in neonatal intensive care. Premature birth, low birth weight and long hospital stay are significant factors for this condition. *Staphylococcus epidermidis*, which is known as commensal, has emerged as the most common cause of devise-related infection. *S. epidermidis* cells are extremely capable of adhering to catheters and forming a multilayered structure called biofilm. Biofilm formation increases the resistance of bacteria to host defense mechanisms as well as to antibiotic therapy.

LL37 is the only member of the cathelicidin host defense peptide family expressed in humans. This peptide produced by leucocytes, cells of the mucosal epithelium and keratinocytes, is considered to be antimicrobial as well as to have anti-biofilm effect.

The aim of this study was to investigate possible inhibitory effect of LL37 on biofilm formation on polyurethane central venous catheter. Biofilm formation was performed in vitro by incubating pieces of catheters in liquid bacterial cultures of the laboratory strain ATCC35984 in the absence and presence of LL37. 1 mg/l and 16 mg/l peptide-concentrations were used. Biofilm was analysed by scanning electron microscopy (SEM).

The biofilm mass was weaker and the bacterial number reduced on the catheter surface at 1 mg/l compared to conditions in the absence of the peptide, as judged by SEM. More bacterial inhibition was found at the higher LL37 concentration. Similar results are obtained with polystyrene microtiter plates.

This study suggests that human cathelicidin peptide LL37 could be a new candidate in diminishing devise-related infections caused by *S. epidermidis*.

Keywords: Coagulase-negative staphylococci (CoNS), CVC, SEM

Identification and disinfection of biofilm forming microorganism in drinking water storage tanks

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The formation and accumulation of biofilms on drinking water storage tank walls is a long-lasting problem in water supply systems.

Our target is to identify bacteria leading to the formation of biofilms in specific locations using new and effective methods and to investigate the effectiveness of certain disinfectants against these biofilms.

A biofilm is an organic film composed of mixed populations (bacterias, fungis, protozoas) and a self-produced matrix of extracellular polymeric substance (EPS). This matrix protects the cells encapsulated in the film and allows communication among them by chemical and physical signalling.

Factors leading to the formation of biofilms include surface consistence, type of microorganisms, and prevailing nutrients. Adapted to specific environments, they can grow on a wide variety of different surfaces and may quickly colonize news habitats.

For the purposes of this project, biofilm samples were taken from different large-scale drinking water storage tank walls and incubated in CASO-Bouillon for 48 hours at 36°C to promote growth of all microbes in the sample. In order to get single colonies of individual microbes, broth samples were plated on blood agar plates for 48 hours at 36°C.

Individual colonies were harvested from blood agar plates and identification of microorganisms was performed using MALDI-TOF (Matrix-assisted laser desorption/ionization-time of flight mass spectroscopy). MALDI is a soft ionization technique allowing the analysis of biomolecules and organic molecules by mass spectrometry,. To this end, a matrix is used to protect the biomolecules from being destroyed by the laser beam needed for the evaporation and to facilite the vaporization and ionization of the biomolecules.

Secondly, we used a microtiter plate biofilm assay to check the biofilm formation capacity of bacterial species identified in the samples described before. The assay employed here is performed in 96-well plates. Cells were grown on microtiter plates for 24 hours at 37°C. Subsequently, wells were washed to remove the planktonic bacteria. Cells remainig adhered to the wells were stained with a dyeand optical density was measured photometrically.

From microbes identified so far, we reproduced biofilms under laboratory conditions. For the growth of biofilms, we put microscope slides in vertical position on Teflon carriers in a beaker at 36°C (optimium bacterial growth temperature) with water and CASO-Bouillon (bacterial nutrition) in continuous agitation. Usually 7 days later, we confirmed biofilm formation by confocal laser scanning microscopy (CLSM), a technique for obtaining high-resolution and three-dimensional images.

Finally, we studied the effect of certain disinfectants against individual bacterial species and biofilms as mentioned before. Additionally, we made qualitative and quantitative assays investigating the disinfectant efficacy against bacterial species (e.g. *Pseudomonas aeruginosa, Staphylococcus aureus*) and against fungis (e.g. *Candida albicans*).

Preliminary results indicate that efficiency of disinfectants against individual biofilms may be evaluated using methods described before.

Keywords: Biofilm; MALDI; Confocal laser scanning microscopy; Antibacterial disinfectants.

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Identification of Small Molecule Inhibitors of Biofilm Formation by *Salmonella* Typhimurium and *Pseudomonsa aeruginosa* and Investigation of Their Mode of Action.

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A major difficulty in the control of *Salmonella* and *Pseudomonas* infections is the fact that these bacteria can form biofilms, in which they are protected against the influence of antibiotics, disinfectants and the immune system. Therefore, the prevention of biofilms could be an effective way to restrict the spread of *Salmonella* and *Pseudomonas.*

We chemically synthesized several libraries of natural product analogues and tested their influence on the biofilm formation of *S.* Typhimurium and *P. aeruginosa* by using a high throughput biofilm assay. In this way we identified different classes of potent biofilm inhibitors for which we delineated structure activity relationships. As a first class of compounds we studied the brominated furanones, which were originally isolated from the seaweed *Delisea pulchra* and which are known to inhibit biofilm formation and quorum sensing in several other pathogens We synthesized a library of twenty-five 1'-unsubstituted and 1'-bromo or 1'-acetoxy 3-alkyl-5-methylene-2(*5H*)-furanones via existing and via new methods. This library was tested for the antagonistic effect against the biofilm formation by *Salmonella* Typhimurium. As an additional model system, we determined the effect of these compounds on the quorum sensing regulated bioluminescence of *Vibrio harveyi*. The length of the 3-alkyl chain and the bromination pattern of the ring structure were found to have a major effect on the biological activity of the 1'-unsubstituted furanones. Remarkably, the introduction of a bromine atom on the 1' position of the 3-alkyl chain did drastically enhance the activity of the furanones in both biological test systems. Secondly, we demonstrated the potential of the (bromo)alkylmaleic anhydrides as a new and chemically easily accessible class of biofilm and quorum sensing inhibitors. As a third class of compounds we investigated the 4(5)-phenyl-2-amino-*1H*-imidazoles. A series of more than 160 N1-substituted and 2N-substitued 2-aminoimidazoles and 100 imidazo[1,2-a]pyrimidinium salts, which are the chemcial precorsors of the imidazoles, were synthesized via our previoulsy established chemistry. A clear relationship was found between the nature of the substitutents of the imidazoles and the salts and their biofilm inhibitory activity against *Salmonella* and *Pseudomonas*. The most active compounds of this series were shown to inhibit the biofilm formation at low micromolar concentrations. A good correlation was found between the activity of the imidazo[1,2-a]pyrimidinium salts and their corresponding 2-aminoimidazoles, supporting the hypothesis that the imidazo[1,2-a]pyrimidinium salts are possibly cleaved by cellular nucleophiles to form the active 2-aminoimidazoles.

We investigated the mode of action of the different classes of biofilm inhibitors by using a broad scope of techniques such as gene reporter fusions, transcriptome analysis, qRT-PCR, mutant analysis and phenotypical assays, which has led to the identification of the target pathways of some of the compound classes. We are currently using our knowledge of the target receptor and the structure activity relationship of the biofilm inhibitors to identify new classes of biofilm inhibitors via a number of receptor-based and ligand-based computational techniques.

Conclusively, we identified several classes of potent *Salmonella* and *Pseudomonas* biofilm inhibitors and made progress in the elucidation of their mode of action.

Keywords: *S.* Typhimurium, *P. aeruginosa*, Biofilm formation, Biofilm inhibitors, Brominated furanones, 2-aminoimidazoles, imidazo[1,2-a]pyrimidinium salts

In search of Broad Applicable, Small Molecule Inhibitors of *Salmonella* Biofilm Formation.

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Salmonella is one of the leading causes of foodborne infections worldwide. A major difficulty in the prevention and treatment of *Salmonella* infections is the fact that *Salmonella* is able to form biofilms on various biotic and abiotic surfaces. Biofilms are multicellular structures, in which the bacteria live in a self-produced gel-like matrix. Within these biofilms, *Salmonella* is protected against a wide range of environmental influences such as desiccation, antibiotics, disinfectants and the host immune system. As such, biofilm formation is an important survival strategy of *Salmonella*, both in- and outside the host. Therefore, the prevention and/or eradication of these biofilms could be an effective way to limit the spread of *Salmonella*.

To identify new *Salmonella* biofilm inhibitors we conducted a high-throughput screening (using the 'Calgary Biofilm device') of a compound library, consisting of > 20.000 small molecules, in search of *Salmonella* biofilm inhibitors which are active at a temperature ranging from 16 °C to 37 °C, and therefore have potential to be used both in- and outside the host.

The library contains a broad range of compounds, selected on their possible drug ability. The compounds have a molecular weight between 200 and 500 dalton and the screening was executed both at 16 °C and 37 °C, with a compound concentration between 20 µM and 50 µM. We aim at identifying compounds that inhibit biofilm formation, but that do not kill the bacteria. In this way, the development of resistance, which is a major drawback of classical antibiotics, is less likely. As such, these biofilm inhibitors could be a sustainable alternative in the production of safe and healthy food.

Out of the 20.000 compounds tested, we identified 144 (0.72 %) possible biofilm inhibitors ('hits'), of which 68 are active at 16 °C, 34 compounds at 37 °C, and 38 compounds are active at both temperatures. Subsequently, the dose-response relationship of the 'hits' was determined, as well as the effect of the compounds on the planktonic growth of *Salmonella*, using a 'bioscreen' (Labsystems). The compounds with maximum biofilm inhibitory activity and minimal effect on planktonic growth, were studied further, both with respect to prevention and destruction of biofilms from *Salmonella* Typhimurium and *Pseudomonas aeruginosa*, at different temperatures (16 °C, 25 °C, 30 °C and 37 °C).

Using these results we identified 9 'lead' families, from which analogues were purchased (± 20 analogues/family resulting in a library of 184 analogues). Using these analogues the "structure-activity relationship" of the 'leads' will be determined, to select and/or optimize the most potent compounds. This will yield several 'lead-compounds'. From these 'lead-compounds' the activity in different *in vitro* and *in vivo* test systems will be determined, as well as the 'Mode of Action'.

Keywords *Salmonella* Typhimurium; *Pseudomonas aeruginosa*; biofilm; small molecule inhibitor

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In situ synthesis of antibiotics inside nanoreactors

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Implant-related infections remain a challenge to medicine. Bacterial infections following orthopedic implant surgery are a major problem entailing complications that are difficult to treat, usually curable only by surgical implant removal, and a source of very high medical costs. We present here the potential use of polymeric enzyme-loaded vesicles to synthesize *in situ* a compound with antibacterial activity. We employ nanometer-sized poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline(PMOXA-PDMS-PMOXA) amphiphilic triblock copolymeric vesicles to encapsulate the enzyme penicillin acylase. The resulting particles are rendered permeable to substrates and products by incorporating the bacterial porin OmpF in their polymeric walls. We show that the obtained 'nanoreactors' are enzymatically active and stable under physiological conditions (pH 7.4, 37°C). Penicillin acylase-loaded nanoreactors were tested on an *E.coli* strain. A strong inhibitory effect against these bacteria was demonstrated.

Keywords nanoreactors; bioactive materials, block copolymers, antibiotics

In Vivo Chlorhexidine Substantivity on Saliva and Biofilm: Influence of Cirdadian Rhythm

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INTRODUCTION:

It has been shown that the Clorhexidine (CHX) retention in the oral cavity depends not only on the adsorption capacity of the product but also on intrinsic factors associated with the antiseptic (concentration, time of application, temperature) and extrinsic factors (presence of teeth, dental prostheses, organic material, and salival pH), all of them can affect its antibacterial activity. However, few authors have performed *in vivo* studies on the antibacterial activity of a single CHX application on the salivary flora, analysing the influence of intrinsic and extrinsic factors.

Objectives:

To assess *in vivo* substantivity of a single mouthrinse with 0.2% Digluconate CHX on salivary flora and undisturbed *de novo* bacterial plaque, differentiating between two times of application: CHX mouthrinse in the morning (CHX-MORNING) and CHX mouthrinse in the night (CHX-NIGHT).

PATIENTS AND METHODS:

The group of study was formed of 10 adult volunteers who wore a individual splint with 6 glass disks for 48 hours to boost the growth of bacterial biofilm (bacterial dental plaque). Saliva samples were collected and 2 disks were removed from each volunteers´ splint at 8, 10, and 12 hours after performing a 0.2% CHX mouthrinse (Oraldine Perio®, Johnson and Johnson, Madrid, Spain) under supervision for 30 seconds at 7 a.m (CHX-MORNING) and 1 a.m (CHX-NIGHT). The saliva and plaque samples were analyzed by epifluorescence and confocal laser scanning microscopes respectively, using BacLight® DEAD/LIVE dual stainning. Counting of live/dead bacteria was carried out in 20 microscopy fields in each saliva sample. Optical sections of 1µm were made throughout the biofilm under the confocal laser scanning microscope in each plaque sample.

The repeated measures ANOVA test was used and comparisons by pairs were made to analyze the intra- and inter-mouthrinse bacterial viability in both mouthrinses, and between them on salivary flora and dental plaque, as well as between both oral ecosystems.

RESULTS:

In the saliva, the percentage of viable bacteria increased with the time in both, the CHX-MORNING and CHX-NIGHT, reaching statistical significance in the majority of constrasts (p=0,001-0,020). The percentage of viable bacteria obtained with the CHX-MORNING were significantly higher than those detected with CHX-NIGHT (p=0,000-0,005). In the bacterial plaque, differences were not detected in the intra- and inter-mouthrinse analysis.

In the CHX-MORNING, the frecuencies of viable bacteria on the salivary flora were significantly higher than on the plaque at 8 hours (56,40 *vs* 35,30%), 10 hours (70,80 *vs* 34,39%) and 12 hours post-mouthrinse (79,80 *vs* 30,72%). In the CHX-NIGHT, the frecuencies of viable bacteria on the salivary flora were significantly higher than on the plaque at 10 hours (38,00 *vs* 28,57%) and 12 hours post-mouthrinse (55,00 *vs* 28,95%).

CONCLUSIONS:

The application of a single 0.2% CHX mouthrinse showed a lower substantivity on the salivary flora between 8 and 12 hours after its application than that found on undisturbed 48-hour bacterial plaque. In the saliva, the bacterial viability recovered progressively and was affected by the time of application of the mouthrinse (DAY or NIGHT); it was more effective when performed at night. In the plaque, bacterial viability remained stable for up to 12 hours after the mouthrinse and was not affected by the time of day the mouthrinse was performed.

These results support the greater physiological dynamics of the salivary flora and the possible reservoir function associated with the structure of de *novo* bacterial plaque.

Keywords: Chlorhexidine; Substantivity; Saliva; Dental Plaque; Circadian Rhythm

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In VivoO Chlorhexidine Substantivity up to 7 Hours After Its Application: Saliva *Versus* Biofilm

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INTRODUCTION:

Numerous authors have investigated the strength and duration of the antibacterial activity of Chlorhexidine (CHX) on the oral microbiota applying *in vitro* methods such as the determination of the minimum inhibitory concentration, bactericidal time kill and various biofilm assays. However, *in vitro* studies usually involve a limited number of species and are created under conditions which cannot guarantee that their composition and structure reproduce the *in vivo* situation. In consequence, there is an increasing interest in developing *in vivo* models which can be examined *ex vivo* without any disruption.

Objective:

To evaluate the *in vivo* antibacterial activity of a single mouthrinse with 0.2% CHX Digluconate on salivary flora and undisturbed *de novo* bacterial plaque up to 7 hours after its application.

PATIENTS AND METHODS:

The study group was formed of 10 healthy volunteers. A special acrylic appliance was designed, with 3 inserted glass disks on each buccal side, allowing for biofilm (bacterial dental plaque) growth. The volunteers wore the appliance during 48 hours and then they performed a single 0.2% CHX mouthrinse (Oraldine Perio®, Johnson and Johnson, Madrid, Spain) under supervision for 30 seconds. Simultaneously, saliva samples were taken and disks were removed at 30 seconds and 1, 3, 5 and 7 hours after CHX mouthrinse. A fluorescence technique (BacLight® LIVE/DEAD stain) image by epifluorescence and confocal microscopes, was used to evaluate bacterial vitality on saliva and plaque samples, respectively. Counting of live/dead bacteria was carried out in 20 microscopy fields in each saliva sample. Optical sections of 1µm were made throughout the biofilm under the confocal laser scanning microscope in each plaque sample. The repeated measures ANOVA test was used and comparisons by pairs were made to analyze the intra-mouthrinse bacterial viability on salivary flora and bacterial plaque, and between both oral ecosystems.

RESULTS:

The mean bacterial vitality in saliva and bacterial plaque under basal conditions was 92% and 80% respectively (p=0.006). At 30 seconds, 1 and 3 hours after CHX mouthrinse the levels of viable bacteria detected in saliva and dental plaque were similar (saliva: 5%, 19% and 43% respectively; plaque: 4%, 16% and 35% respectively). The percentages of live bacteria detected in saliva were significantly higher that those observed in dental plaque at 5 hours (58% *vs* 23%, p=0.002) and at 7 hours after CHX mouthrinse (70% *vs* 30%) (p<0.001).

CONCLUSIONS:

The 0.2% CHX solution showed lower sustained antibacterial activity on salivary flora than on *de novo* bacterial plaque at 5 and 7 hours after mouthrinsing. In accordance with the speculations reported by previous authors, this could express the possible reservoir function associated to the undisturbed young biofilm.

Keywords: Chlorhexidine; Substantivity; Saliva; Epifluorecence Microscope; Dental Plaque; Confocal Laser Scanning Microscope

In vivo quorum sensing and quorum quenching in *Vibrio harveyi* during infection of brine shrimp

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Vibrio harveyi and closely related species (such as *Vibrio campbellii*) are important pathogens in aquaculture that can affect almost all types of cultured animals. The most important problems occur in shrimp larviculture, where mortalities can be as high as 100%. Due to large-scale use of antibiotics, many vibrios have acquired (multiple) resistance, which render antibiotic treatments ineffective. Therefore, alternative treatments to control disease are urgently needed.

One of the alternative strategies that has recently been developed to control infections caused by antibiotic-resistant bacteria is the disruption of quorum sensing, bacterial cell-to-cell communication. and Disruption of quorum sensing has been shown to decrease virulence expression in many bacteria *in vitro* (i.e. in bacteria grown in synthetic growth media). However, microbiologists are becoming more and more aware of the fact that bacteria behave differently in different environments. Hence, the question that arises is whether and how quorum sensing regulates virulence of *Vibrio harveyi* where it really matters: *in vivo* during infection of a host.

In this presentation, we will discuss our current knowledge on the impact of quorum sensing and quorum sensing disruption on the virulence of *Vibrio harveyi* towards different host organisms *in vivo* (i.e. during infection of the host). For instance, we found that AI-2 quorum sensing regulates the virulence of *Vibrio harveyi* towards gnotobiotic brine shrimp larvae (**Figure 1**) and that quorum sensing-disrupting brominated furanones increase survival of shrimp infected with pathogenic vibrios (**Figure 2**). In addition, we will present our latest work on detection of *in vivo* quorum sensing of *Vibrio harveyi* during infection of gnotobiotic brine shrimp (based both on *in vivo* gene expression and activity studies). The use of gnotobiotic animals is quite important in this kind of studies in order to avoid bias caused by the micro-organisms that are naturally present in cultures of higher organisms.

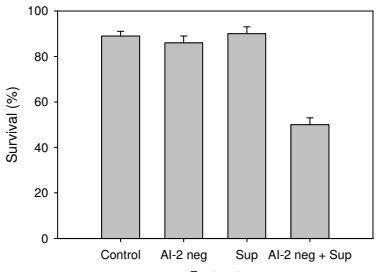

Figure 1: Percentage survival of gnotobiotic brine shrimp larvae after 2 days challenge with a *Vibrio harveyi* AI-2 deficient mutant with and without the addition of cell-free supernatant (as an exogenous source of AI-2). The error bars represent the standard error of four replicates. AI-2 neg: addition of AI-2 deficient mutant; Sup: addition of AI-2 containing supernatant.

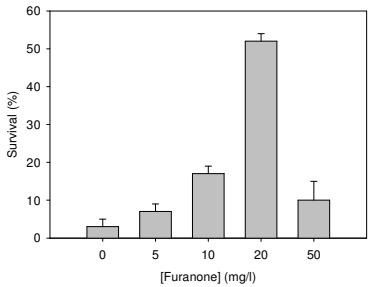

Figure 2: Percentage survival of brine shrimp larvae after 2 days challenge with the virulent pathogen *Vibrio campbellii* LMG 21363, with and without the quorum sesning-disrupting furanone (5Z)-4-bromo-5-(bromo-methylene)-3-butyl-2(5H)-furanone. The error bars represent the standard error of three replicates.

Keywords quorum sensing; vibriosis

Inactivation of influenza virus by photocatalytic reaction with titanium dioxide thin film

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Background: Photocatalytic reaction with titanium dioxide (TiO2) under ultraviolet (UV) light irradiation produces a strong oxidative effect. This oxidizing power has been used for the inactivation of a wide variety of microorganisms in many applications. Although many studies of photocatalytic inactivation of bacteria have been reported, there has been little research of viruses including influenza virus. In this study, we analyzed the inactivation of influenza virus by photocatalytic reaction with TiO2 under UV light irradiation. Additionally, we characterized the effect of the photocatalytic reaction against influenza virus proteins. Here, we are discussing the mechanism of virus inactivation process.

Methods: Human influenza A virus strains (A/PR8/H1N1) was used. Influenza virus was grown with embryonated chicken eggs and concentrated with membrane filter. Subsequently it was purified by sucrose density gradient centrifugation using 10 - 60% linear sucrose gradient. The virus titer was determined by the method of TCID50 with MDCK cells. The photocatalytic reaction was carried out according to ISO27447 with minor modification. ISO27447 is the standard test method for the evaluation of antibacterial activity by TiO2 photocatalysis under UV light. We investigated the most effective condition to evaluate the photocatalytic inactivation of influenza virus as below; (1) The stability of influenza virus with various concentration of BSA, and (2) Inactivation of influenza virus by photocatalytic reaction under different UV intensity. Further, Influenza virus protein after photocatalytic reaction was extracted and analyzed by SDS-PAGE.

Results and Discussion: (1) The stability of the virus in the presence of various concentration of BSA (0, 0.1, and 1.0 mg/ml) was analyzed. The results showed that higher BSA concentration could be stabilized the virus. Inactivation of influenza virus in all BSA concentration photocatalytic reaction under the UV intensity of 0.1 mW/cm2 was observed. On the other hand, influenza virus including 0 and 0.1 mg/ml BSA revealed higher inactivation than virus including 1.0 mg/ml BSA. It was suggested that contents of organics was influenced the effect of photocatalytic inactivation for viruses. From these results, we found the condition with 0.1 mg/ml of BSA had a highest photocatalytic inactivation ratio on virus compared to control. Inclusion of 0.1 mg/ml of BSA might be appropriate for the evaluation of photocatalytic inactivation of virus. (2) We determined the effects of photocatalysis inactivation of influenza virus under different UV intensity (Fig. 1). Photocatalytic reaction even under low-intensity UV (0.01 mW/cm2) inactivated influenza virus effectively. This intensity level is estimated as indoors in the daytime. It means that the photocatalytic reaction could be effective to inactivate viruses even in an actual environment, for example, flooring, indoor glasses and tiles. (3) After the photocatalytic reaction, all protein derived from influenza virus was gradually decreased as UV irradiation time-dependence. All fragments of influenza virus protein were completely digested at 16 hour UV irradiation. Our findings explain that photocatalytic reaction is available for virus inactivation.

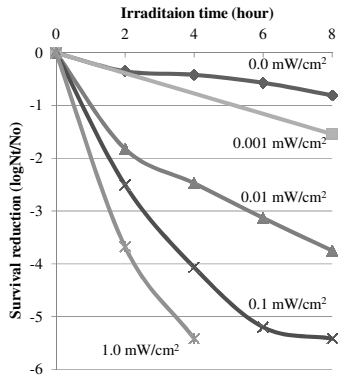

Fig. 1. Photocatalytic inactivation of influenza virus with UVA irradiation (0 -1.0 mW/cm^2).

Keywords; Titanium dioxide, Photocatalytic inactivation, Influenza virus

Influence of a cephalosporin resistance plasmid on the adhesion properties and biofilm formation in *E.coli*

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The formation of biofilm is a universal bacterial survival strategy. Biofilms occur on inert and living support in the natural environment and in industrial installations. This microenvironment leads to the horizontal transfer of genetic material between bacteria by physical contact.

The objective of this study is to evaluate the relationship between the plasmid conferring resistance, surface characteristics and biofilm formation.

The Plasmid conferring resistance to cephalosporin was purified from *salmonella* and transferred by electroporation to *E.coli DH10B* originally unable to form biofilm. The physico-chemical surface properties of the three bacteria (*DH10B*, *Salmonella* and transformant) were estimated by the Microbial Adhesion to Solvents test (MATS) and contact angle measurement (CAM). Cellular density adhered to stainless supports was examined with a scanning electron microscope.

The physicochemical properties of cell surface determined by MATS showed that the *E.coli DH10B* strain was hydrophilic, electron donating and weakly electron accepting, than the *Salmonella* and transformant. Correlation between acid-base properties determined by MATS and CAM was very weak. Adhesion results of images analysis indicate that the transformant and *Salmonella* adhered on the stainless surface, whereas the *E.coli DH10B* does not adhere.

The results of this study indicate that the presence of the plasmid conferring resistance to cephalosporin modifies the microbial surface properties and biofilm formation.

Keywords biofilm; plasmid; surface characteristics

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Influence of feed delivery processes on biofilm formation in liquid feeding systems for pigs.

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In the natural environment, biofilms act as reservoirs for micro-organisms and help preserving ecological balance. On the contrary, in other environments such as hospitals, food-processing industry and breedings, biofilms are not desirable because they constitute a reservoir of contamination very difficult to eliminate. Micro-organisms can adhere to themselves and to surfaces. As a result less than 0.5‰ of micro-organisms are free. The main inconveniences of biofilm are: the constitution of a thick biofilm obstructing the flow of fluids in pipelines, biofilms acting as reservoirs susceptible of containing pathogenic bacteria, and biofilms damaging materials because of the corrosion. In the food-processing industry, the contribution of positive biofilms is envisaged to prevent the development of pathogenic germs such as *Listeria, Salmonella* and *Staphylococcus*. A pre-biofilm, alive or dead, could amplify or prevent the adhesion of bacteria of the same species or of other species. (Ref biblio?)

Our study refers to the development of biofilm in pipes transporting liquid feed for pigs. The liquid feed is prepared in tanks then it supplies troughs. It is possible to rinse pipes with water after each supply of the feed and between two meals pipelines are replete either with feed or with water. Two types of feeds are used for pigs: fermented liquid feed (FLF) or non-fermented liquid feed (NFLF). Fermentation of the feed allows the acidification of the medium and the elimination of enterobacteria providing that the right level of fermentation is achieved (*E.coli*, *Salmonella*). (Ref biblio?)

Table 1 shows which parameters were tested.

Liquid feed	**Liquid filling pipes between 2 meals**	**Pipe material**	**Position**	**Time (days)**
FLF	FLF	HDPE High-density polyethylene	On the top	5
NFLF	NFLF	PVC Polyvinyl chloride	On the bottom, with particles sedimentation	10
	Water			

Table 1: Parameters tested to evaluate differences in adhesion for microorganisms in biofilm

Three technicals are used to determine biofilm composition: firstly by quantifying revivifiable bacteria (total aerobic flora, lactic acid bacteria, yeasts, coliforms bacteria and *E.coli*), secondly through imagery (live/dead coloration observed by epifluorescence microscopy) and thirdly through direct observation (by Scanning Electron Microscopy).

Results show that the only parameter having an influence is liquid feed. Fermented liquid feed eliminates coliform bacteria, meaning they can not exist in biofilm. The population census of revivifiable micro-organisms gives similar results after 5 and 10 days. The analysis of imagery results (after 5 and 10 days) shows the development of the matrix and an increase in the total number of micro-organisms on pipe. These results show that the biofilm is mainly constituted of dead bacteria and that it is quickly established even if it continues to evolve thanks to the incorporation of dead bacteria through the renewal of the medium.

Keywords lactic acid bacteria, biofilm, liquid feed, pig

Innovative High Surface Area CuO Pretreated Cotton Effective in Bacterial Inactivation under Visible Light

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This study presents the first report on bacterial inactivation of *E. coli* by RF-plasma pretreated cotton with high surface area CuO powders compared with non-pretreated cotton textiles. Enhanced adhesion of the CuO due to the RF-plasma treatment was observed. The RF-plasma pretreatment introduces additional binding sites on the cotton with beneficial effects on the *E. coli* inactivation.

The high surface area CuO ($65 m^2/g$) powder was fully characterized and the method of preparation is described in detail. The *E. coli* inactivation proceeded in the dark and was accelerated under visible and sunlight irradiation even at very low levels of visible light irradiation. The effect the RF-plasma pretreatment of the cotton on the binding of CuO, applied light dose, the amount of CuO loading and initial *E. coli* concentration on the inactivation kinetics of *E. coli* is reported in detail. The mechanism of bacterial inactivation is discussed in terms of the semiconductor behavior of *E. coli* under light irradiation. Applying 6 hours Suntest simulated solar light emitting $2x10^{17}$ photons/cm^2/s was sufficient to inactivate completely the bacteria. We estimate that around 7 CFU were inactivated per incident photon. The high number of CFU inactivated per incident photon may be due to three factors: a) the secondary reactions on the cotton leading to *E. coli* deactivation, b) chain reactions occurring during the bacterial inactivation process and c) the hot-electrons produced during the cotton RF-pretreatment. The present findings indicate that low intensity visible light with about 1% of the full solar irradiation had a significant effect on the inactivation of *E. coli* mediated by CuO powders.

Key words: RF-plasma, CuO high SSA, cotton, *E. coli*, visible light

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Investigation of the antimicrobial properties of Ti6Al4V surface after UV light exposure through spectroscopic ellipsometry

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Titanium oxide layers are naturally-occurring thin protective films located at the surface of commercially pure titanium and its alloys. In the medical field, such layers are directly related with the resistant to corrosion of the titanium-based biomaterial surface and govern the interactions with the surrounding media.

Recent studies have shown that the TiO_2 surface exhibits remarkable bactericidal effects after exposure to UV radiation (Gallardo –Moreno *et al*.,2010). The origin of such phenomena has been qualitatively discussed in terms of the physical properties of the irradiated surfaces, which include the emission of energy and changes in surfaces charge occurring during electron-hole recombination processes. We have characterized through spectroscopy ellipsometry the titanium surface before and after exposure to UV radiation. Its optical and electrical properties were quantified in terms of the refractive index, extinction coefficient, and energy band-gap, and these properties were related to the materials bactericidal properties.

Keywords: ellipsometry, antimicrobial surface, surface charge, UV irradiation, titanium.

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Gallardo-Moreno A. M. et al. (2010) Bactericidal behaviour of Ti6Al4V surfaces after exposure to UV-C light. *Biomateria,l* 31:5159-5168.

Irradiation effect on antifungal potential clove essential oil

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The clove (*Syzygium aromaticum*) originates from the "Spice Islands" or Moluscas, Maluku Indonesian province. The clove portion used is the dried bud flower, leaf and stem. The clove has properties anti-irritant, soothing, relief of symptoms associated with motion sickness, nausea and vomiting. The clove essential oil is a clove tree phenolic compound from leaves and flowers (including stems) distillation. When extracted by steam distillation, high Eugenol rates, an aromatic compound are founded. The high eugenol content in essential oil can provide antimicrobial, antiseptic, bactericides, fungicides and fungicidal. The clove also acts as a food preservative due the high eugenol and tannins content. The oil is also used in flatulent colic treatment and some dental procedures. In food alimentation is used as natural flavoring or added in small quantities and still has important applications in perfumery and cosmetics. Spices irradiation is a worldwide process used and this technique is an effective pathogenic microorganisms control providing consumers food security. By the fact cloves not only be used in food, but also as a essential oil raw material this study investigated the cloves different irradiation doses influence on the possible antimicrobial potential oil and there. The aim of this study is evaluate the antifungal potential oil from unirradiated and irradiated clove in the fungus *Guinardia citricarpa* that causes serious damage in orange plantations. The clove samples will be irradiated in ^{60}Co irradiator (Gammacell 220, Nordion Ltd., Canada) at doses of 0, 2.5, 5.0, 7.5 and 10.0kGy.

Keywords: cloves; irradiation; essential oil; *Guinardia citricarpa*; antifungal action

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Is synergy of antimicrobials the effective way of management of resistance among cosmetically significant skin microflora?

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Cosmetic and personal care products (anti-acne, anti-dandruff, anti-odorant, prickly heat talc, etc) use synthetic antimicrobials like Triclosan, Farnesol, Zinc Pyrithione etc or plant based extracts for the anti-microbial 'functional' benefit. The use of single anti-microbial agent would pave way for emergence of resistance in the cosmetically significant skin micro-organisms. To combat the development of resistance and deliver the anti-microbial benefit, a combination of synergistic antimicrobials can be used. The paper reports on the use of synergistic combination of selective synthetic and natural compounds to deliver the desired anti-microbial benefit in these products.

Lactobacillus amylovorus LA 19280 as novel food-grade antifungal agent for bakery products

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Mould spoilage is the main cause of substantial economic loss in bakery industry and might also cause public health problems due to the production of mycotoxins. The reduction of mould growth in bakery products is thus of crucial importance and there is great interest to develop safe and efficient strategies for this purpose. In this study *Lactobacillus amylovorus* LA19280 has been shown to produce a wide spectrum of antifungal compounds active against common bread spoilage fungi. Among the indicator moulds, *Aspergillus fumigatus* and *Fusarium culmorum* were the most sensitive organisms. Several antifungal compounds were found to be present in liquid medium inoculated with *L. amylovorus* LA19280 strain, some of them being reported here for the first time. Wheat doughs fermented with *L. amylovorus* LA19280 had good rheological properties and the breads thereof were of high quality as shown by rheofermentometer and texture analyzer measurements. The results were compared with those obtained using the non-antifungal *L. amylovorus* DSM20531^T strain, a non-acidified and a chemically acidified dough. The quality of sourdough and bread started with *L. amylovorus* LA19280 was comparable to that obtained by using *L. amylovorus* DSM20531 ^{T}. Breads were evaluated for the ability to retard the growth of *Fusarium culmorum* FST 4.05, *Aspergillus niger* FST4.21, *Penicillium expansum* FST 4.22, *Penicillium roqueforti* FST 4.11 and fungal flora from the bakery environment. Bread started with strain *L. amylovorus* LA19280 showed a remarkable antifungal activity in both "*in vivo*" tests indicating that it could be used as novel biocontrol strategy for bakery products.

Keywords Antifungal compounds, bread, *Lactobacillus amylovorus*, food biopreservatives

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Lipopeptide based approach for specific delivery of antitubercular drug isoniazid

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One third of the world's population is believed to be infected with *Mycobacterium tuberculosis* (MTB), the causative agent of tuberculosis (TB). The specific delivery of the recently used antitubercular drug isoniazid (INH), can decrease the side effects and shorten the length of the therapy. Drugs have to be transported through several membranes to reach the target cells. Therefore the increase of membrane affinity of INH was in focus. In this study tuftsin receptor specific lipopeptide was used as a carrier moiety for INH.

The tuftsin derivative (TKPKG) presents similar bioactivity as native tuftsin, a natural phagocytosis stimulating peptide. INH was conjugated to the lysine side chains of the peptide on solid phase [1]. The N-terminus of the peptide was elongated with palmitic acid to enhance the lipophilicity (logP for conjugate = -0.2 (logP for INH = -1.12). The conjugate was chemically characterized by mass spectrometry, analytical RP-HPLC and amino acid analysis. The *in vitro* antibacterial effect of the compounds was determined on *M. tuberculosis* $H_{37}Rv$ bacterial strain. The antitubercular effect of INH was maintained after the chemical modification.

The interaction of INH and its lipopeptide conjugate with a lipid monolayer was followed by surface pressure measurements. Lipid Langmuir monolayers as the most simplified but well defined model of cell membrane [2] was used to assess the cell penetration ability of these compounds.

Nanoencapsulation of the INH-lipopeptide conjugate can enhance the bioavailability and allows the prolonged drug release. The conjugate was entrapped into biocompatible and biodegradable polymeric nanoparticles (PLGA 50:50) as drug delivery system. The encapsulation efficiency of the INH-lipopeptide conjugate was 50-80% depending on the preparation conditions, while the corresponding value was only 1-5% for INH itself.

Oral toxicity studies were performed in guinea pigs (40 mg/kg bw per day) After three weeks oral administration. no significant malformations were observed.

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Keywords lipopeptide conjugates, drug delivery system, isoniazid, antitubercular drug

Listeria monocytogenes biofilm forming ability is not always corelated with benzalkonium chloride susceptibility

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Listeria monocytogenes is a food born microorganism that may cause human and animal disease. Listeriosis is a relatively uncommon disease which occurs primarily in pregnant woman, newborn infants, elderly and immunocompromised but has a high mortality rate. This bacterium's versatility and ability to form biofilms makes it a challenge for food industries since biofilms are recognized as having reduced susceptibility to disinfectant agents when compared to its planktonic counterparts.

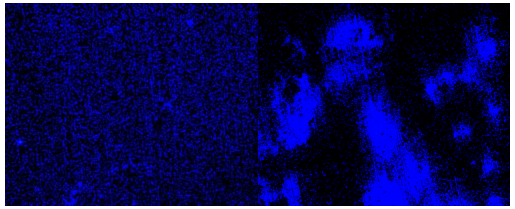
The aim of this work was to investigate the correlation between the biofilm forming ability of *L. monocytogenes* (by direct enumeration of viable cells through plate count, and through visualization by epifluorescence microscopy targeting nucleic acids) and the susceptibility of the biofilms to an antimicrobial compound - benzalkonium chloride (BC).

Thirty two isolates of *L. monocytogenes* from different origins and serovares were used to form biofilms on a surface model of food grade stainless steel. For direct enumeration of the biofilm population, the biofilms were removed from the surfaces by swabbing, and the homogenized suspensions were plated in appropriate culture medium for incubation. The biofilms were also challenged with 25 ppm BC for 5 min and the survivors removed and enumerated, by swabbing and platting. For visualization by epifluorescence microscopy, a nucleic acid fluorescent dye was used to access the area covered by the biofilms, under a 100 × magnification.

The values obtained for biofilm forming ability ranged from 5.8 to 6 Log_{10} CFU/cm^2. The use of microscopy showed a wider variation among strains with values of occupied area ranging from 0.7 to 11.9%. The morphology of the biofilms was found to be distinct among strains even within strains with similar covered areas.

When the comparison of the two methods used to access the ability of the strains to form biofilms was performed, based on the Spearman's correlation coefficient (SCC), a low positive correlation (0.496) was obtained. This may be explained as the two methods used are based on different approaches. On one hand, the swabbing and plating of the biofilms recovers only viable cells and the formation of biofilm clumps difficult to desegregate may occur. On the other hand, the epifluorescence microscopy does not account for the tridimensionality of the biofilms and the stayning of nucleic acids includes non viable cells.

The corelation between the biofilm forming ability of the strains assessed by the two methods and the susceptibility of the biofilms to BC was determined. The value of SCC obtained when correlating susceptibility with log CFU/cm^2 was 0.6, whereas it was 0.05 when correlating susceptibility to BC with microscopy data. Although a significant correlation was obtained between directly enumerated biofilm and Log reduction, the susceptibility of the biofilms to BC can not be reliably explained by the biofilm forming ability of the strains. According to our results, it not possible to accurately predict the response of a strain to BC only based on its biofilm forming ability. Susceptibility testing are thus the more accurate method to measure the potential of the strains to cause recurrent contaminations within food industries.



Keywords: *Listeria monocytogenes*, biofilms, epiflurescent microscopy, benzalkonium chloride, susceptibility testing

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Local prophylactic antibiotics for orthopaedic arthroplasty: understanding antibiotic-loaded bone cement and finding an alternative

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Local prophylactic antimicrobial chemotherapy, in the form of antibiotic-loaded bone cement, is well established for minimising infections arising from surgery to fit replacement hips, knees and other orthopaedic prostheses.

The effect of static elution of antibiotics from bone cement has been studied extensively, but relatively little is known about the effect of wear on the elution kinetics. Wear against the cement surface occurs between the stages of two-stage revision surgery, where an infected prosthesis (e.g. a prosthetic hip) is replaced. During the first stage of the operation, the infected prosthesis and damaged tissue are removed and replaced with an antibiotic-loaded cement spacer, which affords the patient mobility until the second stage of the revision, when the new prosthesis is fitted. In the past it has been presumed that friction between cement sufaces or between cement and bone would increase the elution of antibiotic by abrasion of the antibiotic-containing cement. By using a modified laboratory wear testing rig and high pressure liquid chromatography (HPLC) techniques, we have found that the elution kinetics varied substantially with the type of antibiotic that was added to the cement. In the case of cement loaded with 1.25 % (wt/wt) gentamicin, wear reduced the rate of antibiotic elution into aqueous buffer by 4-16 fold. In contrast, when the cement was loaded with the same amount of daptomycin, wear had a negligible effect on the rate of elution of the antibiotic. Electron microscope analysis of the worn cement surface shows that plastic deformation of the cement leads to a 'sealing' of the surface, thereby reducing the surface porosity of the cement. In the case of gentamicin-loaded cement (where the antibiotic was unevenly distributed with large antibiotic crystals being present) the elution kinetics were markedly affected, moreso than for daptomycin-loaded cement where there was a uniform distribution of small antibiotic crystals.

The increasing use of cementless fixation of orthopaedic prostheses, where the prosthesis is inserted directly into the patient's bone, presents new challenges for local delivery of antibiotics in the absence of cement fixation. In order to develop a system for local delivery of antimicrobials that would be suitable for use with such *cementless* prostheses, we have developed a hybrid organic-inorganic sol-gel coating system to which antimicrobials can be added. In vitro tests have indicated that the antibiotic properties of the coatings are unaffected by doses of gamma radiation that would be used to sterilise prostheses and that elution kinetics are appropriate for delivering antibiotic within a release rate initiating within 15 minutes, i.e. during the perioperative period, when pathogenic microorganisms may enter from the patient's skin or the environment and being sustained for up to 168 h. Preliminary cell culture tests indicate that the coatings have low cytotoxicity and hence are promising candidates for further investigation via in vivo testing.

Keywords orthopaedic surgery; prosthesis; antimicrobial coating, elution kinetics; gentamicin; daptomycin

Minimal inhibitory concentration of nanoparticles-impregnated photosterilisable textiles against *Escherichia coli* and *MRSA*

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Ag-doped and undoped nanotitania prepared by a sol-gel method have been incorporated into common textiles in suspension aided by ultrasound sonication and magnetic stirring. The influence of production parameters such as time required for impregnation, temperature during impregnation and ultrasound power, was examined to determine optimum impregnation. The antimicrobial activity of nanotitania (Ag-doped and undoped) impregnated textiles was tested against *Escherichia coli* and *MRSA*. A relationship between the concentration of nanoparticles and the antimicrobial activity of impregnated textiles was determined. This established to find the minimum amount of nanotitania that is needed for photosterilisation of textiles under the condition of blue and UV light at a power density that mimics those in a typical solar spectrum. Photoirradiation under these conditions enhanced the antibacterial activity of the impregnated textiles, but the efficacy depended on the duration of exposure to such simulated conditions.

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Plasma Deposited Organometallic Antimicrobials

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Over the last decade and a half nosocomial infections have become more and more prevalent, due to an increase in multidrug resistant strains of bacteria as well as rise's in ward overcrowding, and the use of drugs and antibiotics which can leave an individual susceptible to secondary infections. Together these factors have created a more vulnerable hospitalised population. Some of those most at risk are those requiring enteral feeding, long-term catheterisation, central venous catheter's or those suffering from severe burns.

Our group's goal is to develop antimicrobial compounds, with low eukaryotic toxicity which can be polymerised onto surfaces of medical devices and wound dressings in an industrially viable method.

We are currently investigating low temperature plasma deposition of several novel antimicrobial Zinc complexes. A systematic study is being utilised to determine how compound concentration, coating method and plasma pulse delay affect the antimicrobial efficacy of the Schiff base when deposited onto a non woven polypropylene fabric. Coated fabric is tested against the prevalent pathogen's *Staphylococcus aureus* strain 476 and *Pseudomonas aeruiginosa* strain PAO1 using the Japanese industrial standard for antimicrobial coatings

So far we have seen small but significant correlations between pulse delay and the antimicrobial activity of the surface bound compound, and decreases of 2 and 4 orders of magnitude in Colony forming unit count's. Coating with hydrophobic adlayers may decrease a bacterial species ability to form defensive biofilms on the cloth. We are also attempting to elucidate the compounds ability to protect against a pathogenic challenge in vivo using the *Manduca sexta* as our insect model and the pathogen *Photorhabdus luminescens*.

Keywords Antimicrobial; Surfaces; In Vivo

Plasma polymerisation and retention of antibacterial properties of terpinen-4-ol

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A significant body of reports supported by contemporary *in vitro* and clinical studies indicate tea tree oil to be effective alternative to conventional antimicrobials, particularly in the case those organisms that developed resistance against systemic antibiotics. Most bacteria tested were found to be susceptible to tea tree oil at concentrations of 1.0% or less, with minimal inhibitory concentration (MIC) of above 2% reported for commensal skin staphylococci and micrococci organisms, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. In the case of methicillin-resistant *Staphylococcus aureus*, the MICs and minimal bactericidal concentrations (MBC) were identified to range between 0.25-0.31% and 0.5-0.63%, respectively, depending on the origin of the isolates. In its vaporized form, tea tree oil has been demonstrated to be effective against *Mycobacterium avium*, *Escherichia coli*, *Haemophilus influenzae*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*, reducing the incidence of nosocomial infections in hospital patients. Despite promising *in vitro* and *in vivo* research data, many aspects of oil's bioactivity are yet to be explored. In particular, most of the *in vitro* studies and all of clinical research has been performed using liquid or aerosol formulation of tea tree oil.

Recent developments in plasma aided deposition technologies allow for the activation of simple organic molecules without destroying their basic chemical functionality and offer high degree of control over the chemical and physical properties of the coatings fabricated. Such coatings can be deposited without compromising the inherent properties of the substrate material. Immobilisation of molecules with specific functionality are of particular interest since they produce surfaces that are highly specific in terms of their chemical reactivity, and hence can exert direct influence on the behaviour and biological function of cells in its immediate environment.

With biomaterial-associated infections recognised as a major hindrance to the long-term utilisation of most implanted or intravascular devices, the focus of this study was to examine the possibility of using plasma aided deposition to coat three-dimensional solid objects with ultra-thin films characterised by desirable biophysical and biochemical properties, with particular focus on antifouling and antibacterial characteristics. The monomer chosen for the study, terpinen-4-ol is the major constituent of *Melaleuca alternifolia* essential oil believed to be primarily responsible for its biocidal activity against a broad range of human pathogenic bacteria.

Plasma polymerisation was used to deposit thin oligomeric films of terpinen-4-ol on a range of substrates. The coatings were examined in terms of their chemical properties and surface architecture to ascertain the changes in chemical composition as a result of exposure to the plasma field. The antifouling and antimicrobial activity of oligomeric terpinen-4-ol coatings were then examined against such human pathogens as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Staphylococcus epidermis*. The bacterial adhesion patterns were investigated using scanning electron microscopy (SEM), atomic force microscopy (AFM) and confocal scanning laser microscopy (CSLM).

Keywords terpinen-4-ol; plasma enhanced deposition;

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Polypyrrole-coated antimicrobial fabrics

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Polypyrrole (PPy) is one of the most studied π conjugated polymer in materials science due to its electrically conductive properties. It can be easily produced by chemical oxidative polymerization from water solutions of pyrrole. Materials immersed in the solution during the chemical synthesis are coated by *in-situ* polymerization with an even PPy layer. This process allows to produce a wide variety of products (such as textiles) able to transport electrical signals.

Antimicrobial property of PPy have recently been investigated. It is supposed that the partially charged nitrogen atoms of PPy act against bacteria like quaternary ammonium salts, since positive charges (delocalized over several monomer units in the polymer backbone chain of PPy) were produced during the oxidative polymerization.

In this work, cotton fabrics were used as substrates for PPy deposition in order to impart both electrical and antimicrobial properties. Cotton is universally appreciated for its comfort, soft handle, water absorbency, strength and easy maintenance, and PPy deposition does not alter these properties. PPy deposition was carried out in sealed vessels at room temperature for 4 h. The fabric was cut in square samples of 10 cm by side and plunged in 50 ml of aqueous solution of oxidants. The used oxidants were iron(III) chloride (0.066M), ammonium persulfate (0.033M) and iron(III) sulfate (0.033M). The pyrrole was added at concentration of 0.030M. After polymerization, the samples were squeezed, rinsed in water, dried at room temperature overnight and stored at 20°C and 65% RH for at least 24 h before testing.

Antimicrobial activity was evaluated following the EN ISO 20645 procedure using *Escherichia coli* (Gram negative) ATCC 8739. For the lower layer, about 10 ml of nutrient medium (yeast extract agar) free from bacteria were poured into sterilized Petri dishes and frozen. For the upper layer, 150ml of agar were inoculated with 1ml of bacteria working culture ($1–5\times10^8$ cfu/ml). The inoculated nutrient medium was vigorously shaken to distribute the bacteria evenly, then 5ml were poured into each Petri dish and frozen. Round specimens with a diameter of 25±5mm were cut from the PPy coated fabrics and placed in the Petri dishes ensuring that there was a good contact between specimen and agar for the whole incubation period. The Petri dishes were incubated for 18 h at 37±1°C. After the incubation period, the plates were observed. The inhibition zone was negligible for all the specimens: the colonies of bacteria grew around the fabric. Removing the specimen from the agar (as the procedure requires) we observed the absence of colonies under the fabrics (contact zone). Therefore, there is antimicrobial activity on the fabric surface just by contact because the antimicrobial agent (i.e. PPy) is fixed directly to the fabric. Moreover, unlike small molecules, PPy having high molecular weight cannot diffuse. The absence of bacterial growth, even without inhibition zone, may be regarded as a good antimicrobial effect. A low diffusibility ensures no dispersion of active substances in the environment or on the skin. Work is in progress to assess the antimicrobial properties of PPy coated fabrics obtained with different polymerization conditions, also against *Klebsiella pneumoniae* and *Staphylococcus aureus*.

Keywords polypyrrole; antimicrobial textiles

Potential Applications of Targeted-Nanoparticles as Antimicrobial Agents

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The overall goal of this research is to determine the potential for using nanoparticles conjugated with antibodies to specifically target antibiotic-resistant pathogenic bacteria. In the work described, we fabricated core-shell nanoparticles with a silica core and a gold shell using previously described methodology. Destruction of targeted bacteria can be accomplished by heating these particles with near-infrared radiation (NIR), capable of penetrating, but not destroying healthy tissue. We used Atomic Force Microscopy (AFM) and electron microscopy as a means to verify and standardize synthesis procedures to control the size of both the silica core and the gold shell. By controlling growth time, concentration of tetraethylorthosilicate (TEOS), and the catalytic base (ammonium hydroxide), silica core nanoparticles can be tailored to sizes ~30-100 nm. Likewise by varying synthesis time and concentration of gold chloride, the gold shell thickness can also be controlled. Thiol (S-H) chemistry is being used to attach ligands terminating in either amino or carboxyl groups to the gold shell using standard chemical techniques. This allows for proof-of principle experiments to assess targeting and NIR killing effects using *E. coli* bacteria and *E. coli* antibodies conjugated to nanoparticles as a model system.

Keywords gold-silica nanoparticles, near-infrared radiation, antimicrobial, atomic force microscopy, electron microscopy

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Precursor layers do not affect the antimicrobial properties of Ti6Al4V surface after exposure to high energy ultraviolet irradiation

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Introduction: *S. epidermidis and S. aureus* represent the majority of strains causing nosocomial infections on metallic implant surfaces. The ability of both strain to colonize the surface of implants and host tissues is facilitated by several bacterial surface proteins which have a high affinity for extracellular-matrix components. These proteins, termed MSCRAMMs, recognize fibronectin, fibrinogen and albumin [1, 2].

In this scenario, Ti6Al4V is one of the most largely alloys used for orthopedic implant design. Its surface is spontaneously covered with TiO_2 which, in turn, shows photocatalytic response upon irradiation with ultraviolet light. Although traditional TiO_2 photocatalysis has been successfully applied in different environmental areas, this bactericidal technology has been studied upon irradiation with UV light at levels that, in clinical medicine, would induce serious damage to human cells. In addition, taking into account that direct illumination is needed, this methodology cannot be carried out in implanted biomaterials surrounded by tissues. Our group has recently shown that UV-irradiation generates a residual bactericidal effect on the bare TiO_2 surface after turning off the UV lamp, without compromising the excellent biocompatibility of the biomaterial [3] and in this work we will show that this antimicrobial effect is not suppressed when coating the material surface with a precursor layer of proteins, which, in turn, is a closer situation to the *in vivo* context.

Materials and Methods: 25 mm diameter disks of Ti6Al4V were kindly supplied by SURGIVAL S.A. G15-T8 UV lamps, emitting predominantly at a wavelength of 254 nm, were kindly provided by Philips Iberica, Spain. Disks were used without irradiation (control) and after 15 h of UV-irradiation (UV).

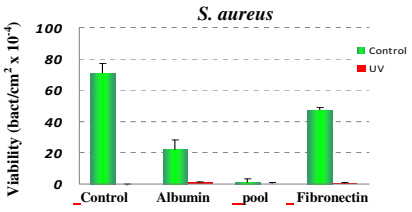
Three gram-positive strains with different EPS-production were used: *Staphylococcus aureus* ATCC29213 (*S. aureus*), *Staphylococcus epidermidis* ATCC35984 (*S. epidermidis*4), *Staphylococcus epidermidis* HAM892 (*S. epidermidis*2).

Different proteins were employed, albumin from human serum, 96-99% (Sigma-Aldrich), fibronectin from human plasma, 0,1% solution (Sigma-Aldrich) and a protein pool from human plasma.

Adhesion experiments were carried out with the help of sterile silicone chambers, fixed to the Ti6Al4V surface by applying a little pressure to its top part. The protein suspension was added to the chamber well and the contact with the Ti6Al4V surface was allowed for different times of 5 and 15 min. After this adhesion time, the protein suspension was removed and added the bacterial suspension for 60 min. Adhesion experiments were carried out in an environmental chamber at 37 ºC and under slight orbital shaking of 20 rpm.

After the adhesion process, the viability of adhered bacteria was evaluated with a standard staining-based method (kit Live/Dead Baclight L-7012)

Results and Discussion: All bacteria deposited on the control surface after the contact time with protein were 100 % viable (green, see Figure), while bacteria attached to the irradiated surface showed an almost complete loss in viability (see figure example on the number of viable bacteria attached to the Ti6Al4V surface at the end of the experiment adhesion with different proteins for strain *S. aureus*). In addition, the number of attached bacteria on the surface with fibronectin is higher than with the other proteins, possibly due to the two fibronectin binding protein FnBPA and FnBPB localized on the bacterial cell surface [1, 2].



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Preparation and Antimicrobial Evaluation of Ciprofloxacin Multivesicular Liposomes

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The aim of the present study was to design an ocular depot delivery system of ciprofloxacin hydrochloride(CIP) using multivesicular liposomes (MVLs) for intraocular injection in order to reduce the frequency of topical administrationa and long-pursued objective in ophthalmology. The multivesicular liposomes bearing ciprofloxacin were prepared by reverse phase evaporation method. For comparison, the conventional multilamellar liposomes (MLVs) containing ciprofloxacin were prepared by film cast method. The formulations were characterized for vesicle size, encapsulation efficiency, and in vitro drug release. The effect of various concentration of α –cyclodextrin (αCD), β-cyclodextrin (βCD) , hydroxypropyl-βCD (HPβCD) on capture volume, particle size and drug release were studied .The encapsulation efficiency of the MVLs (48.70% - 79.49%) was found to be 3 to 6 times higher than MLVs.The results show that the presence of cyclodextrin in aqueous solution has significant effects on the capture volume. The in vitro drug release from various formulations of CIP-MVLs was found to be in a sustained manner in 4-6 days whereas CIP-MLVs released of drug in 20 hours. In vitro antimicrobial effect of these vesicules also was evaluated. Minimal inhibitory concentration (MIC) of the liposomal ciprofloxacin against Staphylococcus epidermis and *Pseudomonas* aeruginosa were significantly (about two fold) lower than free ciprofloxacin. In conclusion, incorporation of ciprofloxacin in MVLs can be enhanced the antimicrobial effects of drug.

Keywords : ciprofloxacin, liposomes, antimicrobial effect

Preparation and Antimicrobial Evaluation of Topical Nanosilver Cream

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A most prominent nano product is nanosilver and the remarkably strong anti microbial activity is a major direction for development of nanosilver products. It is estimated that of all the nano materials in medical, nanosilver application has the highest degree of commercializiation. The aim of this study was to prepare a formulation containing nanosilver particles, which could be applied for wound dressing. Nanoparticles were prepared by using polyethylene glycol 20000 and effect of various conditions on characteristic properties of nanoparticles was evaluated. The minimum inhibitory concentration of nanoparticles against *staphylococcus aureus* and *Escherichia Coli* was determined. The antimicrobial activity of a topical cream containing 0.1% silver nanoparticles was compared to a commercial silver sulfadiazine cream. The results show a high inhibitory effect as compared to commercial cream.

Keywords : nanosilver, antimicrobial effect, topical creams

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Preparation of silver or zinc loaded nanocapsules with core-shell architectures and their application as metal-ion release agents in plastics leading to antibacterial and fungicidal surface properties

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Dendrimers and hyperbranched polymers (HBPs) are able to function as carriers for guests like nanoparticles, metal ions or small molecules. In combination with their low cytotoxic properties, such dendritic branched molecules are already used as nanocapsules for drug delivery. The intra-molecular uptake of nanoparticles into the cavities of the branched molecule structure make sure that a high surface to volume ratio can be realized in the end use application leading to a high bioavailability. The handicap of nanostructure aggregation can be overcome by this approach.

In the case of silver nanopartricles the high specific surface is decisive to realise a constant release of silver ions as active silver species to render plastic surfaces with antibacterial properties. In this manner, we prepared silver nanoparticles with mean diameters of 5 to 10 nm and narrow particle size distribution encapsulated in a dendritic polymer with various core-shell architectures in a novel and robust approach. Beside a long shelf-life of the dispersions, by this approach the compatibiliy of the silver-hybrids can be adjusted to the application in a wide range. This ensures a high dispersivity without leaching of the hybrid additive in a plastic matrix. It could be demonstrated that these dendritic silver-hybrids can be used as effective antibacterial agents in many plastic applications (e.g. thermoset coatings, thermoplastic elastomers, polyamide, polyolefines). Our experimental results have shown that not only the high specific surface area of the nanosilver but also the dendritic carrier polymer itself make a contribution to a high biocide efficiency. Furthermore, the preparation of dendritic zinc complexes based on the same carrier system led to hybrid additives that possess both, antibacterial and fungicidal properties of plastic surfaces, which is of special interest particularly for many medical applications.

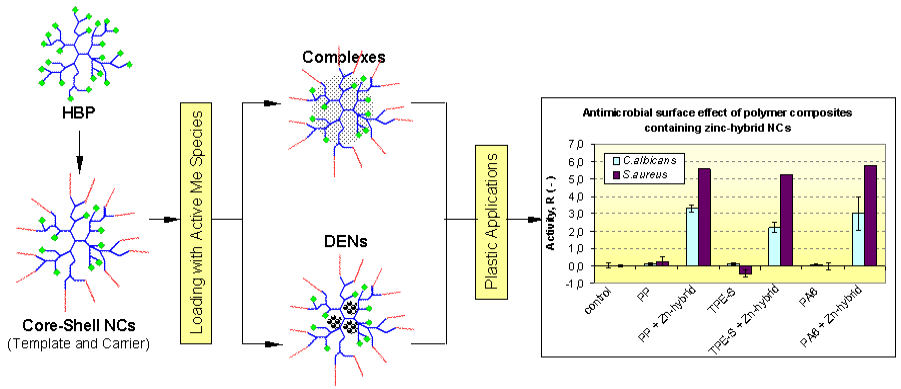

Fig.: Schematic illustration of the preparation process of the silver or zinc loaded nanocapsules (NCs) with core-shell architectures and their application as metal-ion release agents in plastic applications

Keywords dendritic polymers; silver; zinc; plastics; antibacterial; fungicide

Preparation of Syndiotactic Poly(vinyl alcohol)/Poly(vinyl pivalate/ vinyl acetate) Microspheres with Radiopacity Using Suspension Copolymerization and Saponification

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Intoduction

Poly(vinyl alcohol) (PVA) obtained by the saponification of poly(vinyl ester) like poly(vinyl acetate) (PVAc) and poly(vinyl pivalate) (PVPi). There have been some reports on the preparation of monodisperse PVAc and PVPi as precursors for PVA through low temperature suspension polymerization of VAc and VPi. According to the study, to obtain polymer beads with a narrow particle size distribution (PSD), a stable steady state monomer droplets is necessary, and excessive coalescence should be excluded at the growth stage, which may be realized by lowering the polymerization temperature.

The general method for preparing s-PVA is the saponification reaction of P(VPi/VAc) in a solution state; a concentrated aqueous solution of sodium hydroxide is dropped into a completely dissolved P(VPi/VAc)/methanol solution. Some problems arise in its application to the embolization because of its irregular surface and broad PSD. It was reported P(VPi/VAc) microspheres were saponified in aqueous alkali solution contaninig sodium hydroxide, sodium sulfate, and methanol and converted to s-PVA from the surface resulting s-PVA/P(VPi/VAc) skin/core structure microspheres.23) This heterogeneous saponification restricted the reaction on the surface of P(VPi/VAc) microspheres dispersed in aqueous alkali solution and was feasible to preserve the spherical shapes of P(VPi/VAc).

Experimental

Materials

VPi and VAc (Shin-Etsu, Tokyo, Japan) were washed with an aqueous solution of NaHSO3 and water and dried over anhydrous CaCl2, followed by distillation in nitrogen atmosphere under reduced pressure. The initiator 2,2'-azobis(2,4-dimethylvaleronitrile) (ADMVN) (Wako Co., Osaka, Japan; 99%) was recrystallized twice from absolute methanol before use. PVA with a number-average molecular weight of 127,000 and a degree of saponification (DS) of 88% (Aldrich Co., Milwaukee, WI) was used as a suspending agent. TiO2, Au, Ag and Pt nanoparticles aqueous solutions were purchased from Miji Tech. Co., Ltd., Korean. Ti nanopowder was purchased from Aldrich Co..

Preparation of P(VPi/VAc)/nanoparticles microspheres

To prepare P(VPi/VAc) microspheres with radiopacity, the suspension copolymerization approach in the presence of aqueous radiopaque nanoparticles was used. Suspension agent was dissolved in water under a nitrogen atmosphere and with constant stirring, in a 500 ml three-neck round bottom flask fitted with a condenser. After degassing, VPi, VAc, nanoparticles aqueous solution or powder and ADMVN were added sequentially at a fixed polymerization temperature.

Heterogeneous surface saponification of the P(VPi/VAc)/nanoparticles microspheres

The P(VPi/VAc)/nanoparticles microspheres were slowly added to flask during stirring under a nitrogen atmosphere at a fixed saponification temperature. The flask equipped with a reflux condenser, a thermocouple, a dropping funnel, and a stirring device, alkali solution (sodium hydroxide/sodium sulfate/ethanol/water). After the reaction, the reaction mixture was poured into cold water and kept for 1 day to separate and sink s-PVA/P(VPi/VAc)/ nanoparticles microspheres of skin/core structure.

Characterization

The particles size and PSD were measured with video microscopy (VMS) (ICAMSCOPE, SOMETECH, Seoul, Korea). Two hundred microspheres were chosen randomly, and the diameter of each microspheres was measured. Then, the standard deviation (δ) was calculated as follows:

$$\delta=[\sum(d_i-d)^2/(n-1)^{1/2}] \qquad (1)$$

where n is the number of microspheres and and di are d the diameter of each microsphere and the average diameter, respectively. The dispersion coefficient (ε) was calculated as follows:

$$\varepsilon= \delta /d \qquad (2)$$

For ε values less than 0.1, the microspheres can be considered to be monodisperse. The surface morphology of the microspheres was investigated with a scanning electron microscopy (SEM) after gold coating. The microsphere with a skin/core structure were obtained through the control of the saponification time. The skin/core structure of microspheres was confirmed with video microscopy (VMS). Dispersion of nanoparticles in microspheres was observed with transmission electron microscopy (TEM). Radiopacity of microspheres was confirmed with Computed tomography (CT).

Results and Discussion

The P(VPi/VAc)/nanoparticles microspheres were saponified in heterogeneous system, and then the P(VPi/VAc)/ nanoparticles microspheres without aggregates were converted to s-PVA/P(VPi/VAc) microspheres of skin/core structure through the heterogeneous surface saponification. Figure 1 is VMS photoraphs of s-PVA/(VPi/VAc) microspheres with skin/core structure by saponification time. According to increasing of saponification time, skin later of s-PVA/(VPi/VAc)/nanoparticles microspheres was gradually thick.

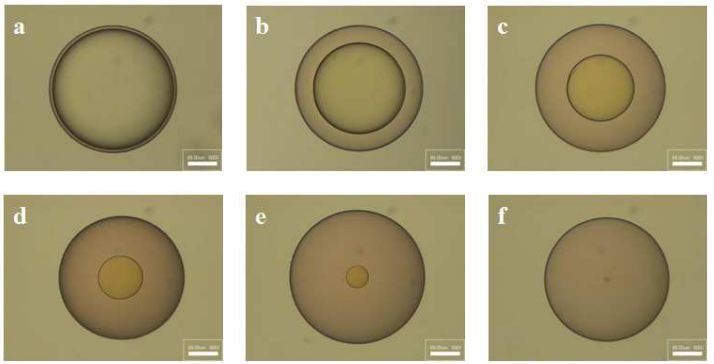
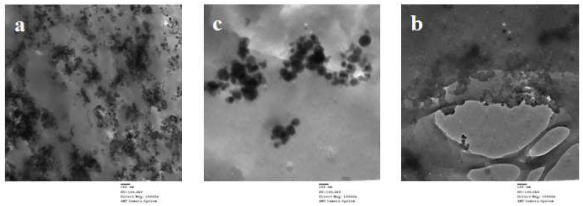


Figure 1. VMS photoraphs of s-PVA/(VPi/VAc) microspheres with skin/core structure by saponification time : a, 24 h; b, 48 h; c, 72 h; d, 96 h; e, 120 h; f, 144 h.

Figure 2 is TEM images of P(VPi/VAc)/nanoparticles microspheres with various nanoparticles. The TEM images provides direct evidence that nanoparticles embedded in the P(VPi/VAc)/nanoparticles microspheres. As shown in Figure 2 (a), although cohesion phenomenon happens a little between TiO2 nanoparticles, TiO2 nanoparticles of P(VPi/VAc)/TiO2 microspheres inside were evenly distributed. As shown in Figure 2 (b, c), Au and Ti nanoparticles aggregated in P(VPi/VAc)/Au and P(VPi/VAc)/Ti microspheres inside. Au and Ti nanoparticles were not distributed evenly. Also, Pt nanoparticles aggregated in P(VPi/VAc)/Pt microspheres inside as in Figure 2 (d). But, Ag nanoparticles of P(VPi/VAc)/Ag microspheres inside were not aggregated.



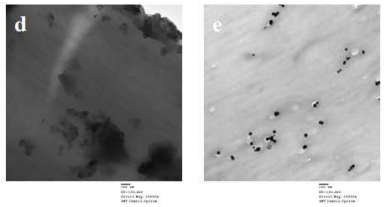


Figure 2. TEM images of P(VPi/VAc)/nanoparticles microspheres with various nanoparticles : a, homo; b, TiO2; c, Au; d, Ti; e, Pt; f, Ag.

Radiopacity of the s-PVA/nanoparticles microspheres was confirmed by CT. Figure 3 is CT photograph of s-PVA/nanoparticles microspheres. The results, The CT photography showed that s-PVA/TiO2 and s-PVA/Ti microspheres have excellent radiopacity.

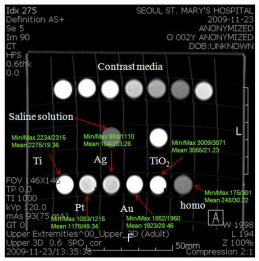


Figure 3. CT photograph of s-PVA/nanoparticles microspheres.

Acknowledgements

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Preparation of Syndiotactic Poly(vinyl alcohol)/TiO_2 Nanocomposites Microspheres Using Suspension Copolymerization and Heterogeneous Saponification

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Commercial poly(vinyl alcohol) (PVA) embolic particles such as Contour™ and Ivalon® have been obtained by the pulverization of a fully saponified PVA sponge. However irregularity of particles size and shape have caused an inflammatory reaction in the wall of the embolized vascular tissue and made it difficult to occlude targeted blood vessels selectively. Therefore, uniform particle size, spherical morphology and stability to human blood should be required.

In this study, to prepare poly(vinyl pivalate/vinyl acetate) (P(VPi/VAc)) microspheres with radiopacity, the suspension copolymerization approach in the presence of aqueous TiO_2 nanopartlcles was used.

Figure 1 is TEM image of P(VPi/VAc)/nanoparticles microspheres with TiO_2 nanoparticles. The TEM image provides direct evidence that nanoparticles embedded in the P(VPi/VAc)/nanoparticles microspheres.

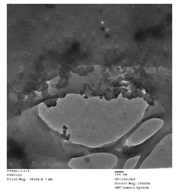

Figure 1. TEM image of P(VPi/VAc)/TiO_2 nanocomposite microspheres obtained by suspension copolymerization.

After the P(VPi/VAc) microspheres with TiO_2 nanoparticles were saponified in heterogeneous system, and then the nanocomposite microspheres without aggregates were converted to s-PVA/P(VPi/VAc) microspheres with TiO_2 nanoparticles of skin/core structure through the heterogeneous surface saponification.

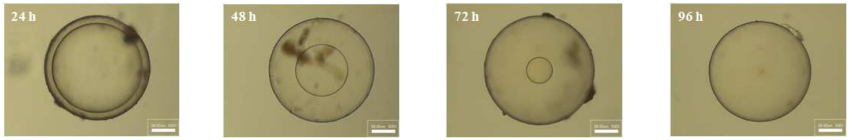

Figure 2. VMS photographs of s-PVA/P(VPi/VAc)/TiO_2 nanocomposite microspheres with skin/core structure by saponification time.

Figure 2 is VMS photographs of s-PVA/(VPi/VAc)/TiO_2 microspheres with skin/core structure by saponification time. According to increasing of saponification time, skin later of microspheres with TiO_2 was gradually thick. After 96 h, core later of the microsphere with TiO_2 was nearly disappeared.

Keywords PVA; TiO_2; suspension copolymerizatoion; radiopacity

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Preventive and curative effect of CSA-13 in different models of biofilms

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[*]C.N. and M.T.-H. equally contributed to this work as first authors.

The preventive effect of a cholic acid antimicrobial derivative, CSA-13, on biofilm formation was tested against 8 strains of *Pseudomonas aeruginosa* (both reference and clinical strains). At 1 µg/mL, an inhibition on the adhesion of the bacteria to an abiotic surface was observed for 4/8 strains with the BioFilm Ring Test® method and the Crystal violet staining technique.

The bactericidal activity of CSA-13 was then studied on a model of young and mature pre-formed biofilm. In planktonic cultures, the minimal inhibitory and minimal bactericidal concentrations of CSA-13 were in the 1-25 mg/L range. In young (24 hours) biofilms the sensitivity to CSA-13 was reduced (half-maximal concentration CSA-13 averaged 88 mg/L) and varied among the 8 strains.

One reference and five clinical strains were tested in mature (12 days) biofilms. The effect of CSA-13 was delayed, some strains requiring 9 days exposure to the drug to observe a bactericidal effect.

CSA-13 permeabilized the outer membrane of the bacteria (half-maximal concentration: 4.4 mg/L). At concentrations higher than 20 mg/L, it also permeabilized the plasma membrane of human umbilical vein endothelial cells.

In conclusion CSA-13 has bactericidal activity against *P. aeruginosa* even in mature biofilms and cationic steroid antibiotics are thus potential candidates for the treatment of chronic pulmonary infections of patients with cystic fibrosis. Considering its interaction with the plasma membrane of eukaryotic cells, less toxic derivatives of CSA-13 should be developed.

Key-words: Pseudomonas aeruginosa, cystic fibrosis, ceragenins, biofilm

References:

Nagant C., Tré-Hardy M., Devleeschouwer M. and Dehaye J.P. 2010. Study of the initial phase of biofilm formation using a biofomic approach. J. Microbiol. Methods. *In press*

Nagant C., Tré-Hardy M., El-Ouaaliti M., Savage P., Devleeschouwer M. and Dehaye J.P. 2010. Interaction between tobramycin and CSA-13 on clinical isolates of *Pseudomonas aeruginosa* in a model of young and mature biofilms. Appl Microbiol Biotechnol. *In press*

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International Conference on Antimicrobial Research (ICAR2010)
Valladolid (Spain), 3-5 November 2010

Protease immobilized active food pack

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Polycaprolactam (PCL) was functionalized on its surface with protease in order to prevent the adhesion and proliferation of food contaminating microorganisms. The protease was immobilized on PCL using covalent binding method. The functionalized surface was characterized by FT-IR, Goniometer and SEM-EDAX. The antibacterial activity of immobilized polymer was tested against gram positive microorganism like *Bacillus subtilis* and *Staphylococcus aureus*, gram negative microorganism like *Pseudomonas aeruginosa* and *Escherichia coli* . Viability of the bacterial cells was tested using a dual staining baclight kit. Slimicidal activity of protease immobilized polymer against above organisms was tested. An attempt was made to study the possible mechanism of action of protease on antibacterial activity and biofilm prevention.

Keywords: Polycaprolactam, Slimicidal, Immobilization.

Quorum sensing as an antibacterial target

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There are two broad strategies for the control of bacterial infections, either (a) kill the organism or (b) attenuate virulence such the infecting organism fails to adapt to the host environment and can be cleared by host defences. Anti-virulence agents offer several potential advantages including expanding the repertoire of bacterial targets, preserving the host microflora and exerting less selective pressure, which may result in decreased resistance. In many pathogens, virulence is co-ordinately controlled via sophisticated global regulatory systems such as quorum sensing. This is usually defined as cell population density dependent gene regulation and is mediated via self-generated extracellular signal molecules. These low molecular weight compounds or 'autoinducers' activate or repress QS target genes once a critical threshold concentration of signal has been reached. The key components of any QS 'module' are the QS signal synthase, the signal receptor and the signal molecule. QS systems thus offer multiple targets for chemical intervention through the blockade of QS signal synthesis, QS signal molecule degradation or the inhibition of QS signal reception. Such targets in conjunction with high throughput screens offer multiple opportunities for the design of synthetic inhibitors and the discovery of natural products for the treatment of infections caused by multi-antibiotic resistant bacteria.

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Recognition of peptidoglycan and β-lactam antibiotics by the extracellular domain of the Ser/Thr protein kinase StkP from *Streptococcus pneumoniae*

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The eukaryotic-type serine/threonine kinase StkP from *Streptococcus pneumoniae* is an important signal transduction element that is involved in the regulation of expression of numerous pneumococcal genes through phosphorylation of various protein targets. We have expressed the C-terminal extracellular domain of this kinase (C-StkP protein), carried out a spectroscopic characterization of its structure and stability and elaborated a three-dimensional computational model. Furthermore, we document by circular dichroism and fluorescence studies that C-StkP binds to synthetic samples of the bacterial peptidoglycan, major constituents of the cell wall, and to β-lactam antibiotics, which serve as molecular mimics of the terminal portions of the stem peptide of the peptidoglycan. This is the first direct documentation of recognition of a minimal PGN unit by a PASTA-containing protein kinase. The collective finding reveals that non-crosslinked peptidoglycan could act as a signal for the function of StkP and point to this protein as an interesting target for β-lactam antibiotics.

Keywords: Pneumococcus, Signal transduction, PASTA domains, Peptidoglycan, β-lactam antibiotics, Protein structure

Self-Decontaminating Surfaces: A Revolutionary Approach to Disinfection

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For decades surface contaminants were considered significant contributors to the total burden via dermal and oral exposure. More recent evidence points to them also playing a role in respiratory infections.

Compared with other infectious agents, when exposed to environmental pressure, the stability of viruses displays a number of unique characteristics. Viruses are not living cells and their environmental stability is mainly based on their three dimensional structure. This produces different inactivation kinetics in the course of exposure to antimicrobials inactivation processes. Some viruses, like enteric viruses for example, are much more resistant to inactivation due to environmental conditions or decontamination processes when compared to enteric bacteria.

Starting in the 19 century, efforts to decontaminate surfaces focused on divert chemicals. Great progress was achieved in the development of disinfectants; yet, this approach retained some built in drawbacks, mostly due to the toxicity of disinfectants or their effect on materials.

Efforts to develop self-decontaminating surfaces were reported in recent years.

This presentation will focus on an innovative technology which is based on an advanced composition of polyelectrolyte and polymer matrices and is capable of inactivating various groups of viruses.

The following viruses, each representing a different group of viral structure and stability, were chosen:

Poliovirus type 1 vaccine strain, an RNA virus with a highly stable icosahedral symmetry.

Swine influenza virus, an enveloped RNA viral particle with helical symmetry.

Herpes simplex virus type 1, an enveloped DNA virus with icosahedral symmetry.

All three can be transferred from person to person through contact with contaminated surfaces.

5 drops of 5 µl from each virus were put in contact with a plastic surface coated with the polymer.

Exposure time varied from 0 to 24 hours.

Influenza and herpes viruses were almost immediately inactivated.

Polio virus infectivity declined by a magnitude of 3.3 during the first 4 hours of contact.

These promising results open up a wide range of possible applications. Further tests are needed in order to learn the inactivation mechanism and to define the D value for each virus in various modes of application.

Keywords disinfection; viruses; surface;polyelectrolite

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Valladolid (Spain), 3-5 November 2010

Shelf-life extension of semi-dried fish Nile Tilapia (*Oreochromis niloticus*) by ultraviolet and infrared irradiations

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Shelf life extension of semi-dried fish, Nile Tilapia (*Oreochromis niloticus*), was investigated by means of ultraviolet (UV) irradiation, infrared (IR) spectrum or a combined UV and IR irradiations for 5, 10 and 15 min. Microbial qualities (i.e. total bacteria, total yeast and mold, *Escherichia coli*, *Staphylococcus aureus*) on the semi-dried fish were examined during incubation at 4 ºC for up to 21 days. Quantitative descriptive analysis (QDA) was also employed to quantify various sensorial attributes (i.e. color, appearance, odor, hardness, taste and texture) of the semi-dried fish product during storage. It was found that the combined irradiation of UV for 7.5 min followed by IR for 7.5 min successfully extended the shelf life of the semi-dried fish up to 21 days. Pathogens on the semi-dried fish were reduced to an acceptable level. All sensorial attributes of the semi-dried fish after 21 days storage roughly remained at the levels obtained just after irradiation. These findings illustrate a possibility of using the combined irradiation of UV-C and IR to improve the quality of the semi-dried fish to the required shelf life.

Keywords peppermint oil; semi-dried fish; Nile Tilapia (*Oreochromis niloticus*); ultraviolet; infrared; shelf life; sensory; microbial qualities

Silver Nanoparticles on Clay Disrupt Membrane Integrity of Bacteria

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Nanohybrids, synthesized via silver nitrate reduction in the presence of silicate clay, exhibit a high potency against bacterial growth. The plate-like clay, due to its anionic surface charges and a large surface area, serves as the support for the formation of silver nanoparticles (AgNPs). The nanohybrid consisting of Ag/silicate inhibited the growth of dermal pathogens including *Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa* and *Streptococcus pyrogens,* as well as the methicillin- and oxacillin-resistant *S. aureus* (MRSA and ORSA). Scanning electron microscope revealed that these nanohybrids were adherent on the surface of individual bacteria. The thin silicate plates provide a surface for immobilizing AgNPs in one highly concentrated area but prevent them from entering the cell membrane. Subsequent cytotoxicity studies indicated that surface contact with the reduced AgNPs on clay is sufficient to initiate cell death. This toxicity is related to a loss in membrane integrity due to reactive oxygen species generation. The hybridization of AgNPs on clay surface is viable for generating a new class of nanohybrids exhibiting mild cytotoxicity but high efficacy for battling drug-resistant bacteria.

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Valladolid (Spain), 3-5 November 2010

Structure-reactivity relations for DC-magnetron sputtered Cu-layers during *E. coli* inactivation in the dark and under light

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This study addresses unreported features for Cu DC-magnetron sputtering on cotton mediating inactivation of *Escherichia coli* K12 (from now on *E. coli*) on Cu-cotton fibers compared to bare cotton fibers. The Cu-clusters were observed to be ~50 nm after 40 s DC-magnetron sputtering and presented a wide size distribution. Cu DC-magnetron sputtering leads to thin metallic semi-transparent grey-brown Cu-coating presenting a moderate hydrophobic behavior as determined by contact angle measurements.

Quantitative in-depth profile for the different Cu-species inside the cotton fibers was determined by 5 KeV Ar^+ etching. Sputtering for 40 s deposited 4.10^{16} atoms Cu/cm^2 (taking ~10^{15} atoms/cm Cu per atomic layer) and this was the threshold amount of Cu necessary for complete bacterial inactivation. This is equivalent to a Cu-loading of 0.060% wt/wt or 3 nm/15 atomic layers. The inactivation of *E. coli* was attained within 30 min under visible light (1.2 mW/cm^2) and within 120 min in the dark. XPS identified the Cu-species on the cotton as a function of the sputtering time. For a longer sputtering time of 180s, the Cu-content was 0.294% wt/wt, and the bacterial inactivation kinetics under light was observed within 30 min, as was the case for the 40 s sputtered sample. This suggests that Cu-ionic species play a key role *E. coli* inactivation. The 40 s sputtered sample allows the highest amount of Cu-sites held in exposed surface positions in the cotton to interact with *E. coli* producing the optimal balance of film thickness, crystallite size and roughness. Confocal microscopy shows the higher rugosity of Cu-cotton fibers compared to bare cotton fibers.

Keywords: Sputtering, cotton, *E. coli*, Cu-ionic, etching, XPS.

Surface treatment for improved inhibition of microbial surface colonization: laboratory to real-world application

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Comparing clinical and environmental bacterial species, we found a strong correlation between multidrug-resistant (clinical) pseudomonad strains and mixed-community biofilms occurring in selected areas in intensive care units. These biofilm communities showed a remarkable ability to rapidly recover after biocide treatment, effectively incorporate test opportunistic pathogens in laboratory experiments, offer increased protection to the incorporated pathogens against antimicrobial treatment at levels that kill suspended cells, and serve as a proliferation mechanism that release cells back to the environment. In a clinical context, these results support the notion that environment-to-patient transfer should be considered a mechanism for the spread of pathogens in addition to the conventional emphasis on patient-to-patient transfer. A similar argument can be made for pathogen transfer in public spaces and contamination / spoilage bacteria in food processing and other industries. In view of our results and the proposed role of environmental surfaces as foci of bacterial proliferation, we evaluated and further developed a bio-static surface protectant for its potential application in industrial and clinical settings and showed its efficacy against a number of test bacteria following a new protocol specifically developed to evaluate inhibitor efficiency on hard surfaces (stainless steel, plastic, glass), in combination with standard laboratory methods as well as scanning confocal laser and scanning electron microscopy. Linen was used as an example of a porous surface and the protectant bound irreversibly to this fabric without significant loss of efficacy after 25 wash cycles in an industrial laundry facility. These findings and tests in food processing facilities, hospitals and public areas suggest that the ease of application, cost effectiveness and demonstrated efficacy with this stable, environment friendly microbial inhibitor that persists for extended periods provides a promising approach. Even though it is mostly impossible to have a 100% prevention of bacterial surface colonization with any of the inhibitors currently available and safe for use, incorporating inhibitors with existing cleaning procedures should greatly improve efforts to mitigate infection and contamination.

Keywords surface protection; biofilms, infection, spoilage

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Synthesis and characterization of Tea tree oil (TTO) microcapsules as antimicrobial agent for footwear applications

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Nowadays, due to the current situation of the footwear industry it is necessary the research on materials and concepts as differentiating elements against competitors and to make products stronger in terms of quality, personal health and safety, or respect for the environment. In this sense, microencapsulation presents a new option for the shoe industry as its application can transform traditionally used materials or products into smart materials or products capable of interacting with feet. For instance, they can improve quality of life by incorporating natural products for foot care such as properly dosed essential oils. The microencapsulation of active substances to be incorporated in different footwear components in order to obtain an "active shoe" presents an opening up of a new way of innovation.

In the face of the growing health concerns more and more people return to the use of natural products that are used in the past and until today had been forgotten. In recent years, interest has grown in natural medicinal products, essential oils and other botanicals, in response to the ever increasing incidence of adverse side effects associated with conventional drugs, and the emergence of resistance to antibiotics, synthetic disinfectants and germicides. There has been particular resurgence of interest in Australian Tea Tree Oil (TTO) which has been employed for its germicidal activity since 1925. The oil is obtained by steam distillation of the leaves of *Melaleuca alternifolia* and was used early the last century to treat various ailments with demonstrated activity against bacteria, fungi and viruses. TTO contains almost 100 components, which are mostly terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and related alcohols. The active component of the oil is though to be terpinen-4-ol, although synergistic effects from other terpenes cannot be excluded. TTO shows broad-spectrum anti-microbial activity, which can be principally attributed to terpinen-4-ol and there are susceptibility data on a wide range of gram-positive and gram-negative bacteria.

The *in situ* polymerization allows the formation of microcapsules containing water-immiscible dispersed phase, with improved mechanical properties and thermal stability. The properties of the membrane depend not only on its chemical structure but also on all the synthesis conditions. The polycondensation of the amino resin occurs in the continuous phase, and the phase separation is linked to the pH and the formaldehyde/melamine molar ratio. In this study a series of melamine-formaldehyde (MF) microcapsules containing TTO was prepared to be applied to footwear materials (lining, insoles, etc…).

Therefore, the aim of this study was the synthesis and characterization of melamine-formaldehyde (MF) microcapsules containing *Melaleuca alternifolia* (tea tree) oil to be applied to footwear materials, prepared by *in situ* polymerization process (O/W). Furthermore, the antimicrobial activity of TTO against four microorganisms: *E. coli, B. subtilis, K. pneumoniae and S. aureus*, as typical microorganisms found in footwear, was analysed.

Keywords: Microencapsulation, antimicrobial activity, footwear, essential oils, Tea Tree Oil, *in situ* polymerization

Synthesis of inorganic biocides made of glass/bioglass powders which contain monodispersed silver/copper nanoparticles

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The emergence of nanoscience and nanotechnology in the last decade presents opportunities for exploring the bactericidal effect of metal nanoparticles. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution. The antimicrobial properties of silver nanoparticles are well-established. Althoug only a few studies have reported the antimicrobial properties of copper nanoparticles, they show copper nanoparticles have a significant promise as biocide agent.

This work is focused on developing a new bottom-up route for making glass/bioglass powder that contains monodispersed silver/copper nanoparticles starting from silver and copper nanoparticles embedded into silicate-aluminiun powders (i.e., kaolin, diatoms). This procedure to fabricate glass/bioglass-nMetal biocide powder open the possibility to design a new family of low cost universal biocide powders with a large spectra of applications: health (disinfection, medical implants), agriculture, food and public areas.

First of all, it was synthesized nanostructured powders such as kaolin-n(Ag, Cu) and diatom-n(Ag, Cu), starting from the corresponding salts and with the following reduction of the metal. All the samples were fully characterized by chemical analysis, XRD, UV-V spectroscopy, TEM. To investigate the antibacterial and antifungal effect of these nanocomposites biocide tests were performed inoculating *Escherichia coli* (Gram-negative bacteria), *Micrococcus luteus* (Gram-positive bacteria) and *Issatchenkia orientalis* (yeast).

Once it was obtained the starting materials, it was synthesized glass/bioglass with a content of metal nanoparticles ranging form 0.5 to 5 wt.%, from different homogeneous mixtures of selected glass/bioglass + silicate-aluminium-n(Ag, Cu). These mixtures were melted at 800 ºC and at controlled atmosphere to avoid the coarsening and oxidation of metal nanoparticles. The evaluation of the bactericide activity of the glass/bioglass-nMetals (Ag, Cu) were performed following a similar protocol as it was carried out with raw materials.

Keywords: Kaolin; Diatom; Glass; Bioglass; Copper nanoparticles; Silver nanoparticles; Inorganic biocide

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Targeting RNA Polymerase σ_{70} factor for Universal Antisense Inhibition in Gram Negative Bacteria

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Antisense strategies that inhibit essential genes or resistance mechanisms in antibiotic resistant bacteria have offered an innovative approach to overcome this globally critical health-threatening problem. Many new structurally modified oligonucleotides with excellent local properties overcome the major limitation in target accessibility and in vivo stability for antisense inhibition, whereas the sequence of their targeting gene varies among different bacterial species, compromising their universal application as sequence-specific inhibitors. We explored bacterial RNA Polymerase (RNAP) σ_{70} factor as a novel drug target for universal inhibition in gram negative bacteria using antisense peptide nucleic acid (PNA) against the start codon of essential gene rpoD (which encodes RNAP σ_{70} factor required for transcription initiation and the sequence of which is highly conserved between bacterial species). A 10 mer PNA-peptide conjugate was screened out for growth inhibition in a sequence-specific and dose-dependent manner at low micromolar concentrations in both antibiotic sensitive and resistant strains of *Escherichia coli, Salmonella enterica serovar Typhimurium, Klebsiella pneumoniae and Pseudomonas aeruginosa*. The 10 mer PNA-peptide conjugate with exellent bactericidal activity at high micromolar concentration rescued cells and female mice infected with single or multiple clinical strains of major pathogenic gram negative bacteria from lesions and deaths. Control PNAs–peptide were much less effective, and sequence alterations within the PNA reduced or eliminated inhibition or bactericidal effects. We report here for the first time specific and growth inhibitory antisense conjugate targeting RNAP σ_{70} factor in live gram negative bacteria. The present result is also the first example of PNA-peptide conjugate showing attractive universal inhibitory potential as conventional broad-spectrum antibiotics, in which possible way the antisense antibiotics might develop into to meet the range and type of usage in future health care.

Key Words Antibiotic resistance;RNA Polymerase sigma factor;gram negative bacteria;antisense;PNA-peptide conjugate

The adhesion control of *Listeria monocytogenes* on food-processing surfaces by silver ion implantation

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Bacterial adhesion to a solid surface is a crucial step in the biofilm process. *Listeria monocytogenes* can adhere to food-processing surfaces, survive and grow over a wide range of environmental conditions such as refrigeration temperatures and consequently cause severe disease. Several strategies have been developed with the aim to decrease the adherence of bacteria to surfaces, namely the silver ion implantation on surfaces. Thus, the aim of this work was to determine the *Listeria monocytogenes* CECT 4031 T adhesion ability onto four types of AISI 304 and AISI 430 surfaces usually used in food industry, restaurant, and kitchens, and simultaneously to evaluate the influence of the thermodynamics aspects on bacteria adhesion on these different surfaces.

Coupons (1 cm^{-2}) were cut from a 1 mm layer of AISI 304 and AISI 430 surfaces (N° 2B, 4 , 6 and 8). The silver ions (Ag^{+1}) were implanted at 200 keV, 1.0 µA.cm^{-2} and a dose of 2.0×10^{16} ions.cm^{-2}. All coupons were cleaned by immersion in 0.2% solution of a commercial detergent for 5 min, followed by immersion in ethanol for 15 min. The coupons were twice rising with ultrapure water and dried at 60 °C. Each strain was subcultured in trypticase soy broth (TSB) at 37 ºC in an orbital shaker (120 rpm), overnight. The cells were then harvested by centrifugation at 9000 rpm for 5 min and washed twice with phosphate buffered saline (PBS 0.1M pH 7). The pellets were resuspended in PBS to an inoculum level of 10^9 CFU.ml^{-1}, determined by optical density. Adhesion assays were performed in sterile 24-well microtiter plates and each well was filled with 970 µl of TSB supplemented with 0.6% (w/v) of yeast extract, 30 µl of cell suspension and the respective coupon. The plates were incubated at 4 ºC for 2 h, with constant agitation at 120 rpm. After incubation, the coupons were washed once with 1.0 ml of minimal medium (MM) to remove non-adherent cells and replaced to a new well and the adhered cells were removed by scrapping on 1.0 ml of MM carefully. The number of viable cells was quantified by colony forming units (CFUs) on trypticase soy agar (TSA). The materials and *Listeria* cells hydrophobicity properties were evaluated through contact angle measurements and using the approach of van Oss and coworkers.

The results showed that the strain used was able to adhere to all materials. It was not found significant differences ($p > 0.05$) between the means of the *L. monocytogenes* adhered cells on the twelve surfaces studied. However, the highest mean value of adherence cells occurred to AISI 304 N° 4 (4.78 ± 0.32 log CFU.cm^{-2}) without silver ion implantation (wi) surface. Moreover, it was possible to observe that AISI 430 N° 8 with silver ion implantation (i) (4.29 ± 0.37 log CFU.cm^{-2}) and AISI 430 N° 4 wi (3.60 ± 0.31 log CFU.cm^{-2}) surfaces presentes the lowest means ($p < 0.05$). Concerning hydrophobicity, silver ion implantation increase the hydrophilicity of the surfaces, except in case of AISI 304 N° 6 ($p.> 0.05$). Furthermore, the results showed that *L. monocytogenes* cells are hydrophilic. Moreover, no correlation was observed between the number of adhered cells and substrate surface hydrophobicity, despite of the highest number of bacteria cells adhered mainly occured on surfaces with highest water contact angle value.

The contact time between microorganism and silver implantated stainless steel surfaces seems not to be enough to confer antimicrobial activity. So, we consider that more studies are necessary to evaluate the effective effect of silver as antimicrobial agent to control the adhesion of *L. monocytogenes* cells and biofilms formation. As future work, we will study the effect of silver ion as antimicrobial different time periods.

Keywords: Silver ion implantation; Food-processing surface; Control adhesion; *Listeria monocytogenes*.

The application of essential oils and silver nano particles for explant sterilization in *in vitro* culture

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The tedious part of plant *in vitro* techniques is sterilizing materials and maintaining aseptic conditions. Ideally the disinfectants must be effective against a wide variety of microbial at low concentration. Specific surface area is relevant for antimicrobial activity in silver nanoparticles (SNPs). Moreover, use of natural compounds such as Essential Oils (EOs) could be used as antimicrobial agent. The main objective of this experiment was aimed to studying the substitution probability of SNPs, thymol and carvacrol as novel sterilization agents in tissue culture of bermudagrass. Sterilization complementary treatments (SNPs, thymol and carvacrol) were applied at different concentrations (100 and 200 mg l^{-1}) and exposure times of 30, 60 and 120 min. According to the results, infection of bermudagrass nodal explant (fungi and bacteria) was controlled successfully by SNPs, thymol and carvacrol. Examination of various concentrations in different exposure time showed that 200 mg l^{-1} SNPs in combination with 100 mg l^{-1} thymol in 60 min were inhibited growth contamination. All treatments were not affected on producing callus and necrosis signs of explants. These novel agents specially SNPs, could be used as an alternative to common chemicals treatment for elimination and control microbial population explant in the *in vitro* conditions.

Key words: Sterilization, bermudagrass, SNPs, Thymol, Carvacrol

The Biofilm proteome: the holy Grail

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Given their importance in various industrially relevant areas and in human health, numerous investigations have focused on the particular physiology of biofilm microorganisms. Thus, it is now well recognized that bacteria present in biofilms behave quite differently from their planktonic counterparts. In particular, biofilm organisms are far more resistant to antimicrobial agents than are planktonic organisms. The mechanisms involved in the resistance of biofilm bacteria to antimicrobials are complex and still not fully understood. One of the hypotheses suggested to explain the increased resistance of biofilms to antimicrobial agents, assumes the existence of significant differences in gene expression. Consequently, a number of genetic and proteomic investigations have been directed at determining the degree to which gene regulation during biofilm development controls the switch from planktonic to biofilm growth. While transcriptome analyses led to the conclusion that a limited number of genes show differential expression in planktonic and biofilm cells, protein-based approaches suggested that many genes are differentially regulated during biofilm development – confirming significant physiological differences between free-living and biofilm bacteria. However, multiple phenotypes were identified according to the substratum nature and the different stages of biofilm development (though a biofilm is not merely a group of planktonic cells of varying ages or stages). This versatitily of the sessile mode of life reflects the bacteria ability to detect local physico-chemistry conditions and cell density, and thereby coordinate group behaviour. Some of these adaptations may explain the high resistance level of sessile organisms but little seem biofilm specific. Recently, it was reported that biofilm organisms may modify the proteome of planktonic counterparts, population named Surface influenced Planktonic (SIP) cells by the group of Volker Brözel. Among proteins that were accumulated by SIPs was 3-oxoacyl-[acyl-carrier-protein] reductase, a protein involved in the production of the autoinducer 3-oxo-C(12)-HSL. These data demonstrate that planktonic organisms are also able to detect the presence of a biofilm in their close environment and to modify their protein pattern in consequence.

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The cationic peptide Melimine and its ability to control in vitro and in vivo microbial colonisation of biomaterials

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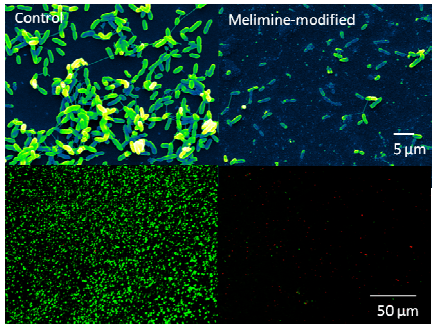
It is envisaged that in our technologically advanced society, every person would have a biomedical device or implant in their life time. However bacterial infections on these life-saving devices have emerged as a major problem. Consequently there is an urgent need to develop biomaterial surfaces which resist bacterial adhesion and colonisation.

We have developed a cationic peptide "Melimine", with excellent broad-spectrum antimicrobial activity. Further this peptide is not cytoxic at active concentrations and is readily heat-sterilizable. In this study we explored the ability of Melimine to prevent bacterial adhesion when covalently tethered on glass and Teflon-FEP using two attachment strategies: non-specific attachment via azide linkers and site-directed attachment via maleimide linker. The resulting surfaces were characterised by XPS and contact angle measurements. The quantity of bound peptide was estimated by a modified Bradford assay. The antimicrobial efficacy of the melimine-modified surfaces against *P. aeruginosa* and *S. aureus* was compared by fluorescence confocal microscopy and scanning electron microscopy (SEM). Up to 40-fold reductions in bacterial adhesion were observed, and a significant increase in the percentages of cells showing damaged membranes. Bacteria exposed to melimine-modified surfaces showed marked changes in cell morphology when observed by SEM. The orientation and concentration of melimine on the surfaces were shown to affect bacterial adhesion. *In vivo* efficacy of melimine-modified surfaces was also demonstrated by a significant reduction in viable bacteria compared with the controls in a subcutaneous mouse model. Coating of biomaterial surfaces with Melimine represents a promising strategy for the prevention of bacterial adhesion both in vitro and in vivo and its performance is influenced by the attachment strategy used.



Keywords cationic peptides; antimicrobial biomaterials, surface coatings

The cationic peptide Melimine controls bacterial adhesion to contact lenses and reduces the production of adverse events in eyes

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Biomaterial related infections have emerged as a serious problem with the increased use of medical implants. Consequently there is a need to develop surfaces which resist bacterial adhesion and colonisation. Melimine, a novel cationic peptide has broad spectrum efficacy in solution, and retains its activity when covalently attached to biomaterials surfaces. In solution, melimine is stable to heat sterilization and is not destroyed by incubating in tears that contain active proteases. Further, repeated passage of these bacteria in sub-MIC concentrations of melimine did not result in an increase in the MIC. Evaluation by electron microscopy has shown that exposure of both *Pseudomonas aeruginosa* and *Staphylococcus aureus* to melimine at the minimal inhibitory concentration (MIC) produced changes in the structure of the bacterial outer surfaces. Melimine was tested for its ability to reduce bacterial adhesion to contact lenses when covalently attached. Covalently linked melimine showed >70% reduction in adhesion of both *P. aeruginosa* and *S. aureus* to contact lenses. Covalently bound melimine killed *P. aeruginosa* cells by disrupting membrane integrity, but the mechanism of killing of *S. aureus* cells did not involve membrane changes. We have also evaluated the performance of Melimine covalently linked to contact lenses in Gram-negative (CLARE) and a Gram-positive (CLPU) models of corneal inflammation. In the model of CLPU, melimine-coated lenses reduced ocular symptom scores and the extent of corneal infiltration ($P < 0.05$). In the CLARE model, melimine-coated lenses showed significant improvement in several parameters including the percentage of eyes with corneal infiltrates ($P < 0.05$).Coating of biomaterial surfaces with Melimine represents a promising strategy for the prevention of bacterial adhesion and consequent sequelae both *in vitro and in vivo* and its performance is influenced by attachment strategy.

The effect of photocatalytic and low energy coatings on brewery surface microbes

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The current trend of improving energy and water saving and reducing chemical loads in the environment support the aim to find novel means to control brewery hygiene. The aim of this study was to find out whether process hygiene in the beverage industry could be improved by applying new coating techniques to process surfaces.

Different photocatalytic titanium dioxide (TiO_2) and hydrophobic low energy coatings were studied both in laboratory and process conditions in order to find out their effect on microbial attachment. In laboratory studies a mixture of three bacteria and one yeast, all isolated from brewery process surfaces, were used in 1-24 h attachment tests. For process studies, coated coupons were mounted to twelve different locations at two breweries. Photocatalytic coatings were in a horizontal position, but low energy coatings were studied also at 10° inclination. Samples were taken by swabbing after 1, 3, 6 and 12 months of mounting the coupons, and attached microbial numbers were determined by culturing.

In laboratory studies photocatalytic coatings were photoactivated with UVA light, but no clear reductions in attached microbial numbers could be seen, except for coatings in which antimicrobial silver (Ag) had been added. These TiO_2+Ag coatings reduced microbial coverage significantly in laboratory studies, and also in some process samples. Low energy coatings were shown in laboratory studies to significantly reduce the area coverage of microbes, but no effect on attached live microbial numbers in laboratory or process studies were seen. In laboratory studies low energy coatings were somewhat better cleanable than stainless steel. The new coatings did not fully withstand process conditions, since part of the hydrophobic coatings had peeled off, most of the antimicrobial Ag had dissolved, and part of the TiO_2 coatings were damaged after approximately 15-months process study. It was also noted that in places where wet and dry conditions alternate, amorphous silica may precipitate on the functional coatings and probably reduce their activity. In addition, the functionality of low energy coatings had changed during the process study, since they had become hydrophilic instead of hydrophobic.

This study showed that several factors influence the functioning of the coatings, and the optimal coatings and conditions for their use were not totally revealed yet. The amount of UVA light is critical for the efficacy of the photocatalytic coatings, and is a limiting factor in process conditions. For functioning of the hydrophobic coatings the inclined position is critical, and possibly the 10° inclination used in process studies was not sufficient for their optimal performance. In addition, this study showed that the durability of the coatings in process conditions should also be considered in further coating development. However, it may need to be accepted that novel coatings will require regular renewal – it just has to be economically feasible.

Keywords: Microbial attachment, process hygiene, titanium dioxide, silver, hydrophobic coatings, brewery microbes

The influence of DC air plasma and cellulase enzyme on the antimicrobial activity of Azadirachtin (neem leaf extract) treated cotton fabric

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The need for antimicrobial textiles is increasing nowadays, due to the detrimental effects of micro-organisms on textiles as well as human hygiene. So it is essential to control or inhibit the growth of these organisms on textile fabrics which turns out to be undesirable for the wearer as well as the textile itself. The natural and ecofriendly, antimicrobial agents for textile applications are gaining interest in recent times. Eucalyptus oil, tulsi leaf, Aloe vera, neem leaf extracts, etc., can be used as a bioactive agent for finishing the textile materials. Neem is known for its environmental compatibility and is harmless against non-target materials, besides being skin friendly and non-toxic. This has significantly increased the use of bioactive agents obtained from neem products in the area of antimicrobial textiles. A large amount of biocides has to be applied on to the fabric to control effectively the microbial growth and sustain the durability of the finish. In the present study, hydrophilicity of the cotton fabrics was increased by DC air plasma and cellulase enzyme treatments to improve the uptake of azadirachtin content in the fabrics. The hydrophilicity of the treated fabrics was determined using dynamic wicking test. The physical and chemical modifications on the plasma and enzyme treated fabrics were analyzed using SEM and ATR- FTIR respectively. The fabrics treated with plasma and enzyme is found to exhibit increased hydrophilicity thereby increasing the uptake of neem leaf extract. Methanolic extract of neem leaf was prepared and applied as an antimicrobial finish for the treated cotton fabrics by pad-dry-cure method. The antibacterial and antifungal properties of neem leaf extract finished cotton fabrics were evaluated for its activity against: S.*aureus*, E.*coli*, *Penicillum, Trichoderma* using standard qualitative and quantitative test methods. The synergetic effect of the two treatments on the antimicrobial activity and wash durability of the cotton fabrics were investigated and the results are discussed.

Keywords: azadirachtin; antimicrobial activity; hydrophilicity; air plasma; cellulase enzyme.

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The influence of electric charge on the biocidal activity presented at the Ti6Al4V surface after exposure to UV radiation

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Ti6Al4V is one the most commonly used biomaterial in ortophedic applications due to its mechanical strength, excellent resistance to corrosion and good biocompatibility. Interestingly, a recent study has detected a significant biocidal activity at the pasivated Ti6Al4V surface after exposure to UV radiation (Gallardo-Moreno et al., 2010). This residual post-irradiation bactericidal effect has been partially attributed to surface charge variations resulting from the interaction of the material with the UV light. We have carried out a detailed study of the titanium alloy electrical properties prior to and after exposure to UV radiation. Classical electokinetic techniques, such as streaming potential and current, were used with caution due to the electric conductivity that metallic samples exhibit in an electric field. Alternative new approaches based on the generation of atomic force microscopy “titration curves” and contact angle “titration curves” were also used to characterize the electric properties of the materials. The results obtained through these later methods were compared with those obtained by classical electrokinetic experiments. A relation between the material surface charge and its biocidal activity after UV exposure is proposed.

Keywords: titanium, surface charge, UV radiation, antimicrobial activity

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The role of antifungals agents on *Candida glabrata* biofilms matrix composition

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Candida glabrata was considered, for years, a relatively non-pathogenic saprophyte of the normal flora of healthy individuals and as no causative agent of serious infection in humans. However, its high mortality rate and its quick spread confirm the opposite. In fact, due to the widespread and increased use of immunosuppressive therapy together with broad-spectrum antifungal treatments, the frequency of mucosal and systemic infections caused by *C. glabrata* has increased significantly. Furthermore, biofilms are described as surface associated communities of microorganisms within an extracellular matrix, generally composed of carbohydrate and proteins. Biofilm formation is an important virulence factor for a number of *Candida* species, as it confers significant resistance to antifungal therapy by limiting the penetration of substances through the matrix and protecting cells from host immune responses. Moreover, little is known about the role of antifungals on *C. glabrata* biofilms. Thus, the aim of this work was to study the role of fluconazole, itraconazole and amphotericin B on 24 h pre-formed *C. glabrata* biofilms and specially on their matrix composition.

A total of 3 *C. glabrata* strains isolated from oral, urinary and vaginal tract were used, as well as a reference strain from ATCC (*C. glabrata* 2001). Biofilms were formed on 12-well plates on RPMI 1640, during 24h at 37ºC and 120 rpm. Then, the antifungal agents (fluconazole, amphotericin B and itraconazole) were added to the previously formed biofilms. After 48 h of action of each antifungal agent, the biofilms were evaluated in terms of total biomass by crystal violet staining and number of viable cells by colony forming units (CFUs). The role of itraconazole on biofilms of the clinical vaginal isolate (*C. glabrata* 534784) was also examined in terms of matrix composition. For this, biofilms were formed in 6-well plates during 24h and, after 48h of exposure to itraconazole, were scraped from the wells and the extracellular matrix was extracted by sonication. Biofilm matrix contents in proteins and carbohydrates were determined using the BCA kit and the Dubois method, respectively.

The results showed that, amphotericin B and fluconazole were able to cause a significant decreased on total biomass and CFUs of *C. glabrata*. However, itraconazole was not able to affect biofilms, except for the clinical vaginal isolate (*C. glabrata* 534784) at 256 µg/mL point concentration, which presented an increase in total biofilm biomass. *C*andida *glabrata* 534784 biofilms matrix exposed to itraconazole (256 µg/mL) presented an increase in proteins content but not in carbohydrate comparatively to the control.

In summary, fluconazole and amphotericin B were able to significantly decrease the pre-formed biofilms of *C. glabrata* strains. Furthermore, the highest amount of total biofilm biomass of the vaginal isolate seems to be due to the increased protein content in its matrix.

Key words: *Candida glabrata*, Biofilms, antifungals agents; resistance

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Urolithins, Metabolites Produced by Human Colonic Microflora, Act as *Quorum Sensing* Inhibitors of *Yersinia enterocolitica* Affecting its Gene Expression

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Urolithins are metabolites produced after the consumption of ellagitannins by the human colonic microflora. These hydroxydibenzopyran-6-one derivatives have beneficial effects as, cardioprotective, antioxidant and anti-inflammatory. *Yersinia enterocolitica* is a mammalian enteropathogen which may cause gastrointestinal syndromes after the consumption of contaminated food or through direct inoculation following a blood transfusion in humans. The three human pathogenic *Yersinia* spp. produce N-acyl homoserine lactone (AHL), *Quorum Sensing* (QS) signal molecules which are involved in the cell population density dependent regulation of virulence, secondary metabolite production, and biofilm maturation. Recent studies have associated the pathogenic activity of *Y. enterocolitica* to specific QS systems. Our objective was to evaluate the effect of the main microbiota-derived metabolites, urolithin-A and urolithin-B, as QS inhibitors in *Y. enterocolitica* and to determine whether or not these metabolites affect the expression of specific genes involved in virulence processes.

It was found that urolithin-A and urolithin-B were effective inhibiting QS of *Y. enterocolitica* when applied at 25 and 50 µg/ml, respectively. The quantification of AHL by LC-MS/MS showed that urolithin-A and urolithin-B (25µg/mL and 50 µg/mL) inhibited the production of *N*-(3-oxohexanoyl)-L-homoserine lactone (3-oxo-C6-HSL) and *N*-hexanoyl-L-homoserine lactone (C6-HSL) in a dose-dependent manner. Urolithin A (80% reduction) showed a higher inhibition than Urolithin B (70% reduction). It was also observed that the tested urolithins affected the gene expression of *Y. enterocolitica*. The data obtained by Real-time PCR showed that urolithins decreased the transcriptional activity of QS regulated genes when low concentrations (25 µg/ml) were used. These findings suggest that very low concentrations (25 µg/ml) of both, urolithin-A and urolithin-B, have an antipathogenic effect against *Y. enterocolitica* and can be used as QS inhibitors reducing the expression of virulence related genes.

Keywords Cell-cell communication; Acyl homoserine lactones; gene expression; virulence

Use of lactic acid bacteria biofilms as biocontrol agents

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A biofilm is an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material (Extracellular polymeric substances-EPS). Also biofilm-associated organisms significantly differ from their planktonic (freely suspended) counterparts. Biofilms have been of considerable directly interest in the context of food hygiene. Biofilms are more resistant to antimicrobial, temperature, Oxygen, protease etc. Biofilms are formed by many microorganisms. Because biofilms formed by pathogen and spoilage microorganisms are resistant to environmental stress, they increase the risk for microbial contamination in food plants. Moreover in recent days, it was discovered that some biofilms have positive properties and it was noticed that these biofilms will be available as biocontrol agents. Use of lactic acid bacteria and their metabolites is the most common and popular in methods of natural protection. Also biofilms are yet another protective agent formed by lactic acid bacteria. On the other hand, there aren't enough studies about use of biofilms as biocontrol agent. In previous surveys, it was mostly focused on antilisterial activity of lactic acid bacteria biofilms. In the other study, it was reported that lactic acid bacteria biofilms can prevent fungal attack. These studies demonstrate the significance of biofilms of LAB in the food industry, so it should be focused on use of lactic acid bacteria biofilms as biocontrol agents with new studies

Keywords: Biofilm, biopreservation, lactic acid bacteria

Utilization of Fish Protein in Submerged and Biofilm Fermentation of *Bacillus subtilis* for Production of Lipopeptide Antibiotic Iturin A

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Different kinds of peptones are being used nowadays as a major ingredient in culture media for cultivation of microorganisms. In this study powdered cod fish meat was used for the production of lipopeptide antibiotic iturin A. Iturin A is an environmentally safe biocontrol agent produced by *Bacillus subtilis* as a secondary metabolite. Generally iturin A is produced in conventional submerged fermentation. Recently, *B. subtilis* has received a huge interest for its nature to develop into biofilm as it shows significantly independent genetic and morphological development in biofilm compared to its planktonic culture. In this study it was attempted to compare the production of iturin A in submerged with that in biofilm fermentation using novel marine fish protein as a medium component. When fish protein was compared with commercially available peptones, it was observed that the cellular growth and iturin A productions were similar to those in the medium containing Polypepton S (originated from soybean) and higher than those in the medium containing Polypepton (originated from casein). When compared interestingly quicker cellular growth and secondary metabolite production was observed in submerged fermentation whereas slower but higher cellular growth and iturin A production was found in biofilm fermentation.

Keywords fish protein; *Bacillus subtilis*; secondary metabolite; iturin A; biofilm

Yeast from urinary nosocomial infection: biofilm and susceptibility to antifungal profile

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Urinary infections caused by yeasts of Candida genus, in hospital environment, are frequent. The object of this work was to evaluate the susceptibility profile of yeasts isolated in patients with urinary infection to antifungal agents, comparing with broth methods microdilution and disk diffusion, and it was evaluated the capacity of these yeasts to form biofilm as well. There were used 98 samples isolated from hospitalized patients. Yeasts were isolated from urine culture with counting inferior to 105 CFU/ml although mixed cultures with bacteria, and cultures collected under the use of probe without previous changing were not selected. Susceptibility tests were evaluated using the following antifungals: amphotericin B, ketoconazole, fluconazole, itraconazole, voriconazole e caspofungin. The biofilm formation was carried out in polystyrene microtitration plate. Even though there were resistant isolates however most of them were susceptible for both methods. In this work, some discrepancies were observed between the susceptibility methods, suggesting that resistant cases for disk diffusion should be confirmed through the reference method (broth microdilution). *C. tropicalis*, had the higher capacity to form biofilm (91.7%) than *C. albicans* (82.5%) and *C. glabrata* (61.3%). In order to avoid biofilm formation, we suggest that the health professionals to be careful during the manipulation of urinary catheters, once the capacity of fungi to form biofilm upon foreign bodies is considered one of the main reasons for the antifungal treatment failure. We believe that the candiduria finding requires more attention and a better monitoring, especially in patients of high risk, considering the importance to avoid systemic infections with high mortality indices. It is also interesting the identification of yeasts isolated from patients with urinary infection, and the performance of susceptibility tests to antifungals as well, in order to avoid empiric therapy, and consequently the emergence of resistant isolates taking into consideration the variability of response to the antifungals evaluated.

Keywords: Candida , Biofilm, Hospital urinary infection

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ZnO nanoparticles coatings for antibacterial and medical applications (ICAR2010)

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Zinc oxide (ZnO) nanoparticles were synthesized and deposited on the surface of glass slides-uncoated and coated with Parylene-using three methods: ultrasound, microwave and microwave plasma irradiation. The structure and morphology of the nanoparticles were studied as a function of the synthesis time and the coating routes. The deposited film was analyzed using characterization methods such as XRD, HR-SEM, EDS, AFM, RBS and optical spectroscopy. Zinc oxide submicron and nanosized crystals with an average diameter of ~100 nm strongly adhered to the glass surface. These methods are fast, simple, convenient, economical, and environmentally friendly. The antibacterial activities of the ZnO–glass composites were first tested against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) strains. A significant bactericidal effect, even in a 0.13% coated glass (wt. %), was demonstrated. At the next stage, we tested the antibacterial coatings for their ability to restrict biofilm formation of these bacterial pathogens. Furthermore, our experiments have demonstrated that antibacterial treatment of ZnO coated glass slides can increase the sensitivity of bacteria cells to two kinds of antibiotics: Chloramphenicol and Ampicillin. A 46% additional reduction in colonies was detected for Chloramphenicol and 37% for Ampicillin due to the cooperative or synergic effect of Zinc Oxide-glass composite and antibiotic treatment.

Keywords ZnO, Coating, biofilm, biomedical materials

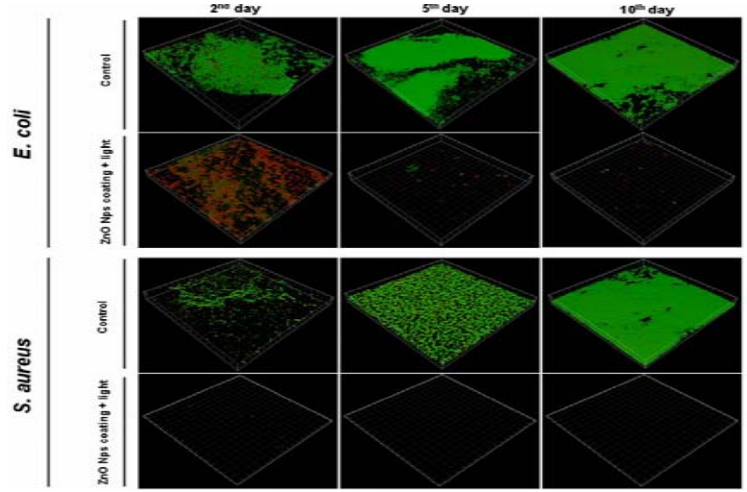

Figure1. Extended antibiofilm activity of ZnO Nps coatings on glass surfaces. (a) CLSM images of *E. coli* and *S. aureus* following biofilms formation over the course of 10 consecutive days on uncoated and ZnO Nps coated surfaces. Green and red staining represents live and dead bacterial cells, respectively.

6. Methods and Techniques - Mechanisms of action - Physics

An Evaluation of Four Different Phenotypic Methods for detection of Metallo-β-lactamase producing *P.aeruginosa* from surgical Patients: A study of Indian Tertiary Care hospital of India

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Purpose: T his study was undertaken to evaluate four different phenotypic methods for detection of metallo-β-lactamase (MBL) producing *P.aeruginosa* in surgical patients.

Methods: One hundred non repetitive isolates of *P.aeruginosa* were collected from General Surgery ward of Chhatrapati Shahuji Maharaj Medical University (earlier KGMU) Lucknow, Uttar Pradesh India, A tertiary care hospital. All the isolates were subjected to susceptibility testing by disc-diffusion assay. Imipenem resistant isolates were determined by disc-diffusion and Minimum Inhibitory concentration(MIC).Screening for MBL production by *P.aeruginosa* was done by Imipenem EDTA combined disc test, Imipenem –EDTA double disc synergy test, Zone Enhancement with EDTA: Impregrnated imipenem and Ceftazidime discs and Modified Hodge Test.

Results: Of one hundred (100) isolates o f *P.aeruginosa*, sixty eight (68) isolates were imipenem resistant by both two methods. MBL screening was done by four phenotypic methods for all imipenem resistant isolates. In this study we found fifty seven (57) isolates were MBL positive by three methods Imipenem EDTA combined disc test, Imipenem –EDTA double disc synergy test, Zone Enhancement with EDTA: Impregrnated imipenem and Ceftazidime discs while fifty four (54) isolates were positive by Modified Hodge Test.

Conclusion: Imipenem EDTA combined disc test, Imipenem –EDTA double disc synergy test, Zone Enhancement with EDTA: Impregrnated imipenem and Ceftazidime discs are equally effective for MBL detection while Modified Hodge Test is less effective for MBL detection because sometimes it give false results. Considering the need to institute correct antibiotics to patients infected with MBL producer and to prevent spread of MBL positive organism, all clinical microbiology laboratories must routinely identify the MBL producers.

Antibacterial mechanism of pentagalloylglucose contained in peony

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Introduction

We have previously reported that 1,2,3,4,6-pentagalloylglucose (PGG, MW940.7) extracted from petals and leaves of *Paeonia lactiflora* Pallas, has a strong antibacterial action. PGG acts on bacteria, but also on virus, enzymes, and toxins, suggesting that the compounds deactivate proteins. PGG also reveals various biological activities such as antimutagenic activity, antiallergic activity and antioxidant activity. These activities correlate with the presence of galloyl moieties of the PGG structure.

In this study, to clarify the mechanism of the bactericidal property of PGG, we tested whether hydrogen peroxide is produced in aqueous solution of PGG similar to catechins[1)] or not. Moreover, to elucidate difference of susceptibility between gram-negative and gram-positive bacteria to PGG, we tested the bactericidal action of PGG against *Salmonella typhimurium* lipopoplysaccharide mutants.

Materials and Methods

PGG was extracted by ethyl acetate from petals of *P. lactiflora* Pallas and isolated by reversed-phase liquid chromatography (DEVELOSIL ODS-HG5 column). Bactericidal activity was estimated as follows. PGG was incubated with approximately 10^5 CFU of bacteria in 10 mM phosphate buffer-140 mM NaCl (pH7.4). After incubation at 35°C for 24 h, serial dilutions were plated and bacterial colonies were counted the following day. Bacterial strains used 10 kinds of gram-positive and gram-negative bacteria. Hydrogen peroxide was measured by the peroxyoxalate chemiluminescence method developed by us[1)].

Results and Discussion

We confirmed by the chemiluminescent method and ESR measurement that hydrogen peroxide is generated by PGG. The hydrogen peroxide generated by PGG is strongly dependent on solution pH. On the other hand, the bactericidal activity of PGG was inhibited by catalase, suggesting that its action was caused by hydrogen peroxide. The bactericidal activity of PGG appears higher against gram-positive bacteria than gram-negative bacteria. This marked difference in PGG susceptibility between the gram-positive and gram-negative bacteria is probably attributable to the barrier function of the outer membrane.

1) Arakawa H et al., Biol Pharm Bull 2004, 27:277-81

Keywords: pentagalloylglucose, *Paeonia lactiflora* Pallas, Hydrogen peroxide, bactericidal activity

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Antibacterial nanofibers based on water-soluble chitosan derivative-Electrospininng and Process Optimization

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Nanofibers containing a cationic polysaccharide, chitosan derivative such as N-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride (HTCC), have been prepared using electrospinning of an aqueous solution of poly(vinyl alcohol) (PVA)–HTCC blends. HTCC, a water-soluble derivative of chitosan, was synthesized via the reaction between glycidyl-trimethylammonium chloride and chitosan. Solutions of PVA–HTCC blends were electrospun. The morphology, diameter and structure of the produced electrospun nanofibres were examined by scanning electron microscopy (SEM). Electrospinning process was optimized unis response surface methodology. The average fibre diameter was in the range of 200–600 nm. Statistical analysis showed that the morphology and diameter of the nanofibres were mainly affected by weight ratio of the blend and applied voltage. The results revealed that increasing HTCC content in the blends decreases the average fibre diameter. These observations were discussed on the basis of shear viscosities and conductivities of the spinning solutions. Microbiological assessment showed that the PVA–HTCC mats have a good antibacterial activity against Gram-positive bacteria, Staphylococcus aureus, and Gram-negative bacteria, Escherichia coli.

Antifungal Assay of thin – Layer Chromatographic Fractions of Seed of *Vitellaria* paradoxa Against Some Dermatophytes

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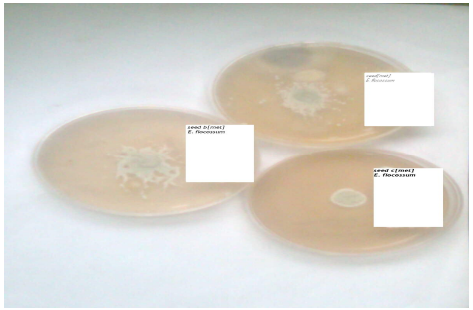
Methanolic extract of seed of *Vitellaria paradoxa* was partially purified by thin – layer chromatographic technique into corresponding fractions.Five active components with distinguishing colours were obtained from the analysis when viewed in UV254nm.The effects of the fractions on some dermatophytes were determined using the ditch method of media dilution technique.

The Minimum Inhibitory Concentration **(MIC)** and Minimum Fungicidal Concentrations **(MFC)** were determined using the broth dilution method. The activities of the extracts were compared with those of standard antibiotics.The fractions showed varying degree of activity against the test organisms between 0-100%. In general all fractions exhibited fungistatic activity against the organisms at concentrations varying from 6.25mg/ml to 250mg/ml, however fungicidal activity was observed at high concentrations than for the **MIC** assay.

Phytochemical analysis of the components revealed the presence of steroids, Saponins, tannins, reducing sugars, flavonoids, general glycosides and Anthraquinones.The possible use of the seed of *Vitellaria paradoxa* as a drug of choice in the treatment of fungal infections and it's incorporation into orthodox medicine to alleviate the problem of drug resistance are discussed.

Keywords; Dermatophytes, *Vitellaria paradoxa.*



Antimicrobial mechanism of monocaprylin on Gram positive and Gram negative bacteria

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Introduction

Fatty acids and their monoglyceride esters are toxic towards a broad range of microorganisms. Caprylic acid is a medium-chain (8C) fatty acid found in milk and coconut oil, and is FDA approved as a food grade chemical. It is therefore of interest as a non-toxic preservative in food. Although the antimicrobial properties of many monoglyceride esters are well described, the exact mechanism of action is not known. The aim of the present study was to investigate the antimicrobial mechanism of monocaprylin, the monoglyceride ester of caprylic acid.

Methods

The antimicrobial effect of caprylic acid and monocaprylin was first compared by measuring the MIC and LC_{50} after exposing *Escherichia coli* and *Staphylococcus xylosus* to the compounds for 1 hour. The cause of cell death by monocaprylin was then investigated further: Its effect on cell morphology was visualised by atomic force microscopy (AFM), and membrane integrity was visualised by propidium iodide staining. Membrane permeabilisation was studied further in a model system of lipid vesicles made from *E. coli* lipid extracts, where the permeability of vesicles to calcein was measured during exposure to increasing concentrations of monocaprylin. Integration of monocaprylin in the bacterial membrane was also investigated by forming supported lipid bilayers, and subsequently measuring the adsorption of monocaprylin into the bilayer using quartz crystal microbalance (QCM), and imaging the lipid bilayer by AFM before and after exposure to monocaprylin.

Results and Discussion

The LC_{50} of caprylic acid was 10 mM and 3 mM for *E. coli* and *S. xylosus*, respectively. The toxicity of monocaprylin was substantially higher than its free fatty acid. LC_{50} was 5 mM for E. coli and 1 mM for *S. xylosus*, although both organisms required a concentration of 9mM to kill all the cells, which was consistent with the MIC observed. Exposure to monocaprylin concentrations higher than MIC resulted in instantaneous permeabilisation of the cells, and effects on cell morphology were visible on *E. coli* but not *S. xylosus* cells. Based on these results, we hypothesise that monocaprylin integrates with and destabilises the cell membrane, whereas the cell wall remains intact. This hypothesis was further supported by the adsorption of monocaprylin in lipid bilayers measured by QCM, and the distortion of the bilayer evident in AFM images. Interestingly, much higher concentrations of monocaprylin were required to permeabilise lipid vesicles compared to cells, suggesting that the interaction of monocaprylin with lipids alone is not sufficient to destabilise the membrane.

Antimicrobial mechanisms of action of *benzyldimethyldodecylammonium chloride*

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Biofilm resistance to biocide is increasin becoming a serious problem with resistant bacteria being often difficult to eradicate or even to treat. This brings huge economic and environmental implications as well as public health problems. Biocides, with their broad spectrum of usage, seem to be a good vehicle to control or prevent undesirable biofilm formation. For a rational design of anti-biofouling measures, it is vital to understand the mechanisms of biocide activity as well as the bacteria mechanisms of resistance.

In this study, the action of the biocide benzyldimethyldodecylammonium chloride (BDMDAC) was assessed using the Gram-negative bacteria *Pseudomonas fluorescens*. The word biocide is a generic term used to describe a chemical agent with antiseptic, disinfectant or preservative activity against microrganisms. Biocides differ from antibiotics in their lack of selective toxicity. In fact, they have multiple biochemical targets and have been used over the years in a diversity of situations. The selected cationic biocide belongs to the family of quaternary ammonium compounds which are surface-active agents. These are known to be membrane active agents with its target site predominantly at the cytoplasmic membrane (inner membrane) in bacteria and at the plasma membrane in yeast. Although BDMDAC is thought to act by disrupting cell membranes, little is known about its detailed mechanism of action. To assed the biocide action against *P. fluorescens* various methodologies were used: Growth inhibitory activity- minimum inhibitory concentration; Antimicrobial Tests with Planktonic Cells-kill curve; Assessment of membrane integrity - Propidium iodide uptake; Physicochemical characterization of bacterial surfaces; Zeta potential of the suspension of cells; Potassium (K+) titration; Scanning Electron Microscopy (SEM); 1-D electrophoresis.

The minimum inhibitory concentration (MIC) was found to be 20 mg/L of BDMDAC and the minimum bactericidal concentration (MBC) was 10 mg/L. At MIC no viable and culturable cells were detected. However with the application of BDMDAC at 10 mg/L there are still viable cells. BDMDAC induced significant changes on cells surface hydrophobicity and morphology. Cells incubated with BDMDAC were analyzed by SEM and compared with untreated cells (control). Cells treated with 20 mg/L are less bulky and their membrane seems to be rougher, wrinkled, deformed. These results are in agreement with potassium titration results where it is possible to observe an augmentation of K+ concentration in outer cells environment with the increase BDMDAC concentration. Additionally, the results obtained from the zeta potential assay demonstrate a -31.17 mV value for control cells and -21.03 mV for cells at MIC concentration, indicating the existence of interactions between the cationic antimicrobial agent and the cell surface. Regarding 1-D electrophoresis, no major differences were detected concerning the loss or the over-expression of outer membrane proteins (OMPs) associated to bacterial resistance (resistance OMPs).

In conclusion, BDMDAC seems to interact with the cytoplasmic membrane causing changes in membrane structure and function manifested by phenomena like disruption of the membrane and loss of membrane integrity with consequent leakage of essential intracellular constituents.

Keywords biocide; antimicrobial action; *benzyldimethyldodecylammonium chloride*, quaternary ammonium compounds.

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Bacterial bioluminescence as a tool for monitoring the real-time activity of antimicrobial agents

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Traditional microbiological techniques are used to provide reliable data on the rate and extent of kill for a range of bactericidal compounds including disinfectants. European Standard test protocols for disinfectants specify obligatory contact times of 5 minutes (to a minimum of 1 minute) to assess bactericidal activity (EN 1276:1997, EN 1040:2005) or 60 minutes (to a minimum of 5 minutes) to assess sporicidal activity (EN 13704:2002). These tests are designed to assess the efficacy of disinfectants in practically relevant conditions, however such techniques provide very limited data regarding the initial rate of kill of fast acting disinfectants over short time domains. In addition, European Standards for surface disinfection testing (EN 13697:2001) specify an obligatory contact time of 5 minutes (with a minimum of 1 minute) followed by rigorous recovery protocols. While these standards are well suited for providing endpoint viability data they provide no insight into the spatial distribution of the sample or the impact of differing surface materials.

We describe the application of recombinant bacterial reporters expressing the *Photorhabdus luminescens lux* operon as whole cell biosensors capable of rapidly reporting the impact of disinfectants in suspension tests and *in situ* on a range of surface materials. The *lux* genes required for bacterial bioluminescence are arranged in a single operon, *luxCDABE*. Since the operon encodes not only the luciferase but the enzyme complex responsible for the synthesis of the substrate, all that is required to produce light in a recombinant aerobic bacterium is the expression of the *lux* operon. The reaction consists of the oxidation of reduced flavin mononucleotide ($FMNH_2$) and the substrate with the subsequent emission of light in the blue-green range (490nm). $FMNH_2$ is a product of bacterial electron transport and the coupling of bioluminescence to bacterial metabolic activity has afforded a unique means of studying the effect of antimicrobial compounds on bacteria, in real-time.

Escherichia. coli Nissle 1917 was selected for this study since following transformation with plasmid pBBR1-MCS2-lite encoding the full *P. luminescens lux* operon, it proved to be a bright, stable and consistent bioluminescent reporter. The reporter was used to assess the efficacy of Electrochemically Activated Solutions (ECAS), a novel fast acting biocide alongside sodium hypochlorite in the absence of organic soiling, or in the presence of either foetal bovine serum or defibrinated horse blood. The use of bioluminescent *E. coli* Nissle 1917 allowed five readings of light intensity to be taken every second, offering true real-time insight into the metabolic state of the bacteria. The impact of varying concentrations of ECAS and bleach was detectable within milliseconds of treatment and the impact of differing levels and types of organic loading was assessed. These detection rates are precluded by traditional microbiological methods.

A standard inoculum of the bioluminescent reporter was deposited onto 25 x 25mm coupons of a panel of test materials representing a range of surface textures and porosity. The surfaces were treated with ECAS and the light output from the target bacteria monitored over time *in situ* using a low light photon camera. Without intervention bioluminescence on surfaces persisted for up to 8 hours. Following treatment, the decline in light output was detected immediately on less porous surfaces, however on surfaces where the bacterial inoculum was able to soak in, successful decontamination was less consistent.

The use of bioluminescent bacteria as whole cell bioreporters allows rapid assessment of the relative efficacy of fast acting disinfectants and the efficacy of antimicrobial surface treatments. Moreover, the application of this technology may enable further elucidation of the mechanism of action of disinfectants by allowing the investigation of time domains precluded by traditional microbiology and allow conclusions to be drawn about the suitability of materials to decontamination within a specified environment.

Keywords bioluminescence; electrochemically activated solutions; whole cell biosensor

Bacterial cell shape determining factors of *Campylobacter* as potential drug targets

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Campylobacter jejuni is the most frequent causative agent of enteric diseases in many countries. The lack of vaccines and emergence of multidrug resistant forms necessitates the development of alternative antibacterials targeting this important pathogen.

One remarkable feature of this bacterium is its ability to change cells shape from a typically spiral (or rod) form to a coccoid form (CF). Despite some contradictory data, the results of a number of studies suggest that this is a degenerative form of the bacterium. Despite being non-viable, the CFs may play a role of biofilm formation. However, the mechanisms of CF formation remain unclear.

In this study we have identified and investigated various putative *Campylobacter* shape-related genes using site-directed mutagenesis and gene expression assays. The gene selection was partially based on sequence similarity to cell shape-controlling genes found in other bacteria. Surprisingly, in contrast to those bacteria, some of these genes appeared to be essential for *Campylobacter*. One of these genes was found to be upregulated in conditions stimulating CF formation.

In addition, dynamics of CF formation was investigated. The results suggest that this is an active genetically regulated process, and not just a result of bacterial degenerative degradation in unfavourable conditions. The finding therefore supports a possibility to develop novel antibacterial drugs targeting the genes and their products involved in bacterial cell shape changes.

Keywords Campylobacter, coccoid forms, gene regulation, biofilm formation

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Bacterial evaluation of Palm "in nature" processed by e-beam

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The palm can be obtained from various species of palms, but the peach palm has aroused great interest by producers, because it has characteristics of earliness and hardiness, creating a palm of great quality and differentiating it from other palm for its yellowish color and sweet flavor. Food irradiation has been used as treatment to ensure microbiological safety of these products to avoid foodborne illness. Their combined use with minimal processing could increase the safety and quality of vegetables minimally processed. The aim of this study was to evaluate the effect of radiation by electron beams in bacteriological control in fresh heart of palm. The microbiological analysis of fresh peach palm *in natura* showed that the radiation beam using an electron beam irradiator (Radiation Dinamics Co. model JOB 188, New York, USA), promoted the reduction of microbial load, helping to increase the microbiological safety of food. Podemos concluir que a dose de 1.5kGy mostrou ser apropriada para a irradiação do produto estudado, pois diminuiu significativamente a quantidade de bactérias encontradas, elevando a qualidade do alimento. It was observed that the dose of 1.5kGy reduced the amount of bacteria and raising the quality of peach palm *in natura*.

Keywords: food irradiation; microbiology contamination; Palm "in nature"

Bacterial protein secretion as a target for novel antibiotics: discovery of small-molecule SecA inhibitors

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Antibiotic resistance is an escalating problem in the chemotherapy of bacterial infectious diseases. Bacteria resistant to almost all of the available antibacterials have been identified, creating an urgent need to identify novel antibacterial targets and leads with new mechanisms of action. In this context, we have focused on the bacterial preprotein translocating ATPase SecA as a prospective target for antibacterial drug discovery.

A large numbe r of bacterial proteins are active in extracytoplasmic locations and have to be transported from the cytoplasm (i.e. where protein synthesis occurs) to their final destination. A major pathway for protein translocation across and integration into the bacterial membrane is provided by the Sec-dependent secretion pathway. The driving force for the protein translocation reaction is provided by SecA. This essential peripheral membrane ATPase is present in a wide variety of Gram-negative and Gram-positive bacteria but has no mammalian homologue, which makes it an ideal target for therapeutic intervention.

The well-characterized *E. coli* SecA (ecSecA) protein was chosen as target protein to provide proof-of-principle that SecA small-molecule inhibitors can be identified via high-throughput screening (HTS). Because the intrinsic SecA ATPase activity is very low, we have generated an ecSecA mutant with elevated intrinsic ATPase activity for the development of a colorimetric HTS assay in 384-well format. The assay was automated and will be applied for screening of a 25000-compound library in order to identify ecSecA inhibitors. Such molecules can serve as important lead scaffolds for the development of a novel class of antibacterial drugs.

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Bioinformatic identification and laboratory evaluation of anti-infective drug targets in *Pseudomonas aeruginosa*

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The Bioinformatics for Combating Infectious Diseases (BCID) Project is developing a computational pipeline to aid identification of anti-infective drug targets, focusing on proteins predominantly found in pathogens and absent from non-pathogens. There is growing interest in disrupting bacterial virulence mechanisms, thereby "disarming" pathogens as opposed to targeting essential cellular functions. This approach will be more likely specific to pathogenic bacteria and may be subject to less selection for drug resistance.

The pipeline includes modules for evaluating the phylogenetic distribution, functional annotation, genomic context, and subcellular localization prediction for a given bacterial protein. High priority protein targets with known 3D structure (including those recently determined in collaboration with structural genomics consortiums) are subjected to *in silico* docking procedures to identify possible interacting small molecules from libraries of novel compounds. Also, existing drugs are being screened to detect possible drug repurposing opportunities. Since some of the most successful antibiotics target RNAs, we are also developing approaches to identify novel essential RNAs and RNA factors required for the expression of virulence genes in pathogenic bacteria.

Our methods are designed to be broadly applicable to any microbial pathogen, but initial efforts are focused on *Pseudomonas aeruginosa* – an opportunistic pathogen that is noted for its intrinsic antimicrobial resistance. Both drug targets and drug leads are being initially assessed in a *Caenorhabditis elegans* infection model. Three of 13 (23%) drug leads tested to date have sucessfully been protective in *C. elegans*. Since non-targeted drug screening has reported success rates in the range of 0.25%, this suggests that improved bioinformatics based drug target screening represents a promising direction to improve anti-infective drug discovery.

Keywords anti-infective; drug target; *Pseudomonas aeruginosa*, bioinformatics

Biophysical investigation of the influence of surfactin on the structures of cyclic antimicrobial decapeptides

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Antagonism of antimicrobial action represents an alternative survival strategy for cohabitating soil organisms. We observed that the biosurfactant surfactin (Srf) from *Bacillus subtilis* have antagonistic actions against gramicidin S (GS) from *Aneurinibacillus migulanus* and tyrocidines B and C (TrcB and TrcC) from *Bacillus aneurinolyticus.* The propensity for direct molecular interaction between the antagonistic peptide pairs was observed using electrospray mass spectrometry (ESMS), circular dichroism (CD) and nuclear magnetic resonance (NMR).

Srf formed complexes in solution with GS that are stable under ESMS conditions, but not with Trc B and C. However, far UV-CD absorption spectra indicated that Srf induced changes in secondary structures and/or higher order self-assembled structures of both GS and Trcs. The first interaction involved the influence on the exposure/orientation of the D-Phe^4 and Orn^2 residue in GS structure. The second resulted in the reorientation of the hydrophobic/hydrophylic regions of the Trcs possibly related to a shifting of their NH-protons signal as also indicated with ^{1}H-NMR. Diffusion orientated NMR (DOSY) showed that Srf and GS formed homo-oligomers of 5-6 units. NMR studies strongly indicated the molecular interaction of Srf and GS involved the orientation of the D-Phe^4 and Orn^2 residue in GS. However, the addition of Srf to GS did not affect the diffusion coefficient of GS indicating the formation of hetero oligomers as observed with ESMS.

Keywords: Antibiotic peptides, surfactin, gramicidin S, Tyrocidines, antagonism, NMR, CD, ESMS

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Characterization of four natural inhibitors targeting the bacterial translational apparatus

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The properties and the prospective applications of four natural compounds, selectively targeting the translational apparatus, are critically evaluated and compared. Three of these inhibitors (GE81112, GE82832 and GE107558) had been identified some years ago, within a library of natural products of microbial biodiversity using an HTS specifically designed for this purpose. The fourth inhibitor (G1) results from a chemical modification of furfural, obtained from sugarcane bagasse.

All four compounds inhibit translation both *in vitro* and *in vivo*. However, unlike the three secondary metabolites of *Actinomycetes*, which selectively inhibit bacterial translation, G1 inhibits both bacterial and yeast translation. Accordingly, while the anti-microbiological activity of GE81112, GE82832 and GE107558 is restricted to bacteria, G1 has a broad spectrum of activity directed against both Gram positive and Gram negative bacteria and fungi, including pathogenic species.

Fast kinetics analyses and standard *in vitro* tests and of the individual steps of the translation pathway have identified the targets of the four inhibitors, suggesting rather unique mechanisms of action for each of them. With the exception of GE107558, which binds to the 50S subunit, the other three molecules bind to the 30S ribosomal subunit. G1 and GE81112 were shown to inhibit translation initiation, the latter proving to be the most effective and selective inhibitor of the 30S P-site, while GE82832 and GE107558 inhibit the translocation step of elongation, albeit with different and novel mechanisms of action. The topographical localizations of the inhibitors on the target ribosomal subunits have been identified by standard chemical probing experiments and/or by preliminary X-ray crystallographic studies. Overall, our results indicate that the three *Actinomycete*-derived inhibitors represent very useful tools for investigating the mechanism of specific steps of translation and might be developed into effective antibacterial agents; G1 represents a promising wide-spectrum antibiotic suitable for effective therapeutic use.

Comparative analysis of the activity of MDR pumps in *Salmonella enterica* and *Pseudomonas aeruginosa* using methods of potentiometry and fluorescence spectroscopy

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Multidrug resistence (MDR) pumps is one of the main reasons of antibiotics resistance in *S. enterica and P. aeruginosa* cells. We used electrochemical and spectrofluorimetric methods to assay the pump activity in these cells. Accumulation of ethidium (Et^+) by the cells was registered electrochemically using the selective electrode. Simultaneously accumulation of Et^+ was assayed using fluorescence spectroscopy: the increase in fluorescence was observed because of the dye binding to DNA. In parallel potentiometric measurements of the accumulation of tetraphenylphosphonium (TPP^+) ions in the cells were performed. Results of our experiments revealed that Et^+ ions easier than TPP^+ penetrate the OM of *S. enterica* cells. Effects of RND-family MDR pump inhibitor phenylalanyl-arginyl-β-naphtylamide (PAβN) and the outer membrane (OM) permeabilizing compounds EDTA and Polymyxin B were studied. At high concentrations PAβN not only blocks the activity of MDR pumps but also triggers depolarization of the plasma membrane. Starved and permeabilized cells are the most susceptible to the depolarizing activity of PAβN. We demonstrated that the temperature, the intensity of aeration and the composition of medium differently affect the RND-family pump activity in *P. aeruginosa* and *S. enterica*. In absence of nutritives in the media *S. enterica* RND-family pumps effectively extrude indicator compounds but the presence of glucose increases the efficiency of cell envelope barrier to lipophilic compounds. Our results indicated that in formation of the envelope barrier to lipophilic cations contribution of AcrAB-TolC pump is higher than that of LPS layer of the OM. In contrast to *P. aeruginosa* cells, the alternative efflux pumps in *S. enterica* fail to compensate the loss of the major pump AcrAB-TolC. Experiments with *tolC* ir a*crB* gene mutants indicated that defects in both the OM and the PM components of AcrAB-TolC pump almost equally contribute to the loss of cell envelope barrier to lipophilic compounds.

Keywords multidrug resistance; pump activity assay

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Developing Electric Cell-Substrate Impedance Sensing method to study *Chlamydia pneumoniae* infection in HL-cells

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Chlamydia pneumoniae is a worldwide pathogenic bacterium that is difficult to eradicate completely from the human host. Normally it has a two stage developmental cycle with an infectious elementary body (EB) and a dividing reticulate body (RB) stage (1). The complexity of these stages makes *C. pneumoniae* infection difficult to monitor in real time and thus a challenging target for drug development. Another big challenge for assay-development is the intracellular nature of *C. pneumoniae*.

Conventional label-dependent methods to monitor chlamydial infection *in vitro* are based on highly laborious and time-consuming fixed cell cultures which can be used to interpret results only from isolated time points (2).

In view of this, we have aimed to develop an easy, non-invasive, label-free, "hands off" -method to follow the host cell exit process of *C. pneumoniae* in real time and to study the effect of selected anti-chlamydial substances on the time and rate of host cell exit of *C. pneumoniae*. Electric Cell-Substrate Impedance Sensing (ECIS) is a label-free method based on the measurement of the electrical resistance produced by cells when they are attached into golden electrodes. This method is suitable to track the attachment and spreading behaviour of mammalian cells (3) and thus it can be used to monitor also the detachment of host cells caused by intracellular pathogens exiting the host (4).

The assay development stage included optimizing the amount of mammalian host cells to form a suitable and reproducible uniform cell layer displaying steady impedance, the beginning and the end being the most important stages to analyze. Different coatings and host cell concentrations were tested. However, as the uncoated electrodes provided higher quality parameters, reached same impedance values, represent a cheaper choice then the protein coatings, saves time and labor - the uncoated electrodes were chosen over the protein coated electrodes.

The optimization also included recording the infectivity of selected two *C. pneumoniae* strains without centrifugation, as the normal procedure includes centrifugation to enhance infection rate. The infectivity of the host cells (HL-cells) with CWL-029 and K7 decreased 20 fold and 100 fold respectively. These results are in line with the literature [5]. Based on this result the CWL-029 strain was chosen for the further studies.

The developed method was proven to work well when the amount of *C. pneumoniae* reached high multiplicities of infection. The starting amount of 2 MOI of *C. pneumoniae* produced reliable changes in the impedance of host cell layer. These results indicated the suitability of using ECIS as a promising new label-free, noninvasive and highly informative tool to monitor the course of *C. pneumoniae* infection. Results showing the behavior of the *C. pneumoniae* infecton when exposed to natural compounds found active in our earlier studies (6) will also be presented. The method gives a possibility to follow the host cell toxicity profiles of the compounds during the same assay.

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Keywords ECIS; *Chlamydia pneumoniae*; CWL-029

Development of a high throughput screening (HTS) assay for inhibitors of bacterial motility

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Flagellar motility has been recognized as an important virulence factor in numerous bacterial pathogens. In addition, flagellins have been identified as Toll-like receptor ligands that could trigger inflammatory responses in the host. Therefore, motility is an attractive target for new anti-infective drugs. *Vibrio cholerae*, the causative agent of Asiatic cholera expresses a single polar flagellum powered by sodium motive force. Expression of motility in this pathogen is required to establish infection, disseminate along the gastrointestinal tract and initiate the formation of biofilm communities. Motility is a highly complex phenotype involving numerous genes and regulators. It requires the synthesis and export of the flagellum, the flagellar motor, energy coupling and the function of numerous chemosensory pathways. Here we present an HTS assay developed in 384-well format to identify compounds that inhibit motility. The assay is based on the standard swarm agar test and compounds that specifically inhibit motility can be readily distinguished from those that exhibit antibacterial activity. A screen of a 10,000 compound chemical diversity set produced several hits that preferentially inhibited motility. Out of the primary hit list, four compounds were identified that inhibited motility with IC_{50} values ranging from 1.9 -14 µg/ml and showed no toxicity. Since inactivation of motility by mutation does not impair growth in broth, we selected one compound that inhibited motility but had no effect on growth rate for further studies. This compound inhibited motility in conventional swarm agar plates but did not prevent the assembly of a wild type flagellum. Treatment of *V. cholerae* with this compound had no effect on protease production and expression of the toxin co-regulated pilus but diminished the production of cholera toxin and biofilm formation. Interestingly, *V. cholerae* grown in the presence of this compound in alkaline media was sensitive to the protonophore carbonyl cyanide m-chlorophenylhydrazone (CCCP) indicating that under this condition the Na^+ pumping NADH:quinone oxidoreductase was not operative. These results suggest that this compound might inhibit flagellar function by affecting sodium motive force. Taken together, our results clearly validate the presented HTS assay for identifying compounds that specifically inhibit bacterial motility. Furthermore, the assay can be readily applied to any pathogenic bacteria in which motility has been shown to play a role in infection. This work was supported by funding from Southern Research Institute, the NIH Molecular Libraries Probe Production Center Network (U54 HG005034) and Research Grant AI081039 from the National Institutes of Health to A.J.S.

Keywords High throughput screening; bacterial motility; *Vibrio cholerae*; cholera

Development of a liquid medium assay for screening antimicrobial natural products against marine bacteria.

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With the aim of searching for new natural compounds with antibacterial activities for therapeutics, biofilm control or biological control of terrestrial and marine pathogens, marine organisms have been shown to be a promising source of originality in terms of chemical structure or mechanism of action (Mayer et al., 2009). Particularly micro-organisms including phytoplankton, cyanobacteria and fungi represent a noticeable source of bioactive primary and secondary metabolites with an increasing number of original compounds isolated over the years (Blunt et al., 2009). In the marine environment, a need for new antibacterial compounds against shellfish and fish pathogens has arisen in the field of aquaculture. Also, knowledge of such antibiotics is of primordial importance for the understanding of their role in marine ecology, i. e. relationships between micro-organisms.

In this study, we focused our research on lipophilic antibacterial compounds. Indeed, using solvent extraction of wet biological matrices, an array of compounds with a large range of lipophilicity have been identified in micro-algae and shellfish during the past 20-30 years. For the screening of such compounds, the agar-diffusion assay on petri dishes is a classical antibacterial test. Nevertheless, this simple method presents numerous drawbacks, such as large consumption of test compounds, low throughput, and subjectivity of measurements.

Here we describe the development of antibacterial assays in a liquid medium using a Bioscreen system. This apparatus consisting of an incubator coupled to a micro-plate reader allows the observation of bacterial growth kinetics, leading to the determination of the growth rate of each bacterial strain cultured in a repeatable, miniaturized and high throughput manner. The first step in developing this bio-assay was to determine the tolerance of selected bacterial strains to solvents miscible with aqueous culture media. A panel of bacterial species was selected for this study, mainly marine bacteria of the genera *Vibrio* (*V. crassostreae*, *V. gigantis*, *V. mytili* and *V.rotiferianus)*, *Vagococcus, Carnobacterium* and *Photobacterium*. Different concentrations of methanol, ethanol and DMSO were tested to insure that they did not interfere with bacterial growth and the reading of the bio-assay.

The effects of solvents on the bio-assay are presented together with the comparison of this bio-assay with the classical agar-diffusion method on petri dishes, with a range of commercial antibiotics.

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Dynamics of Antimicrobial Peptides by Neutron Scattering

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Numerous increasingly realistic simulations of the molecular dynamics (MD) of antimicrobial peptides and their biomembrane interactions have been performed during the past 5-10 years. The utility of such simulations for 'rational' drug design depends critically on input parameters quantifying short- and medium-range molecular interactions (~0.5 to 5 nm) derived from a variety of structural and spectroscopic techniques. Energy-resolving neutron scattering techniques have already been used extensively to characterise the dynamics of globular and fibrous proteins [1], but cognate work on biologically and biomedically important oligopeptides has been initiated only recently [2]. Quantitatively, the object of quasielastic (QENS) and inelastic (INS) neutron scattering experiments is to measure double-differential cross-sections from which dynamic structure factors $S_{inc}(Q,\omega)$ and $S_{coh}(Q,\omega)$ for incoherent and coherent scattering can be extracted ($\hbar Q$ = momentum transfer, $\hbar\omega$ = energy transfer). By virtue of the point-like nuclear scattering, together with much scope for H/D contrast variation, QENS and INS are a unique source of spatiotemporal information on the mobility of protons and protonated groups in parameter regimes overlapping largely with those of MD simulations.

In this contribution, we report results from QENS and INS work in progress on the dynamics of two 15-residue oligopeptides: (i) a hybrid cecropin-mellitin peptide (CM15, cecropin(1-7) – mellitin(8-15)) prepared at Porto *via* step-by-step synthesis and characterised by state-of-the-art biochemical and calorimetric techniques [3]; (ii) the pore-forming peptide gramicidin in D_2O-hydrated phospolipid model membranes (DPPC and DMPC bilayer stacks). In H_2O- and D_2O-hydrated CM15, we have focused on the side chain dynamics which is dominated by 5 lysine and 13 methyl groups. We characterise the evolution of their proton m.-sq. displacements $\langle u_p^2 \rangle$ from an essentially harmonic regime at cryogenic temperatures to the gradual activation of anharmonic and soft-mode interactions towards 300 K. As in similar work on polypeptides (such as collagen and keratin), we are observing three rather than two proton mobility regimes in hydrated CM15. The gramicidin DPPC/DMPC systems exhibit much more complex behaviour. We observe two freezing transitions (from immobile to mobile) discriminating the onset of mobility at different length scales for the lipid acyl chains and the hydration water in between the membrane stacks. Inclusion of gramicidin lowers the temperature of the gel-crystalline liquid transition, and raises the freezing temperature of hydration water in inter-membrane layers.

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Keywords Neutron scattering; peptide molecular dynamics; cecropin-mellitin; gramicidin

Effect of the presence of detergents on the response of microbiological methods for antibiotic detection in milk

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The aim of this study is to analyse the effect that the residues of the detergents used in the cleaning and disinfection of milking parlours has an the response of the microbiological methods for the detection of antibiotics in milk. Antibiotic-free milk samples were spiked with a selection of commercial detergents (5 acid detergents, 5 alkaline detergents, 2 disinfectants products and 5 domestic dishwasher detergent). The spiked samples were analysed by 3 microbiological inhibitor test (BRT® AiM, Delvotest® MCS and Eclipse® 100). None of test was affected by the presence of acid detergents in the milk samples, as all the obtained results were non-violative (negative) for all the concentrations used in the test. In the case of the alkali detergents and disinfectant products, violative (positive) results began to be observed when relatively high concentrations of detergent were present in the milk. The domestic dishwasher detergents showed different results depending on the product and the method used. The variability of the responses indicates that a more thorough study into the presence of detergents in milk and how they interfere with the antibiotic detection methods is needed.

Keywords: detergents, antibiotics, milk, microbiological methods.

Elucidating the structure-function relationship of a bacterial GTPase in DNA replication through random mutagenesis

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ObgE is an essential bacterial GTPase and its role in ribosome biogenesis makes it a potential new therapeutic target. In addition to ribosome assembly, ObgE is involved in various other cellular processes including DNA replication. It has been proposed that ObgE promotes cell survival during DNA replication when replication forks are stalled. To analyze the structure-function relationship of ObgE with regard to sensitivity to stalled replication forks, an error prone *obgE* overexpression library was constructed and subsequently screened for sensitivity to the replication inhibitor hydroxyurea (HU). HU inhibits class I ribonucleotide reductase which upon treatment results in a decrease in intracellular dNTP levels and finally in replication fork arrest. Five mutant *obgE* alleles that caused an increased sensitivity to HU when overexpressed, were identified. Sequence analysis revealed 1 to 5 amino acid changes in each of the selected mutants. Overexpressing proteins carrying each point mutation separately, led to the identification of four residues within ObgE that are responsible for its role in replication fork arrest. Interestingly, three of these residues are located in the conserved 'switch II' region, which is important for the conformational switch of ObgE upon GTP binding.

Keywords bacterial GTPase, error prone library screening, hydroxyurea

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Evaluation of mycobacterial acetyl-CoA carboxyltransferase (ACCD6) as a potential target for new tuberculostatics

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Mycolic acids, the main component of mycobacterial cell wall, are crucial for cell survival and virulence. They are of particular interests, since their biosynthesis is the target of the most powerfull antibiotics such as isoniazid, ethionamide or pyrazinamide. Their biosynthesis is controlled by two fatty acid synthases: FAS I and II. Acetyl coenzyme-A carboxyltransferase is a key enzyme that provides substrate for reactions controlled by FAS I/II. As shown by *in vitro* studies, protein encoded by *accD6* (Rv2247) gene placed in FASII operon could play a crucial role in mycolic acids biosynthesis, providing malonyl-CoA - the building block for FAS I/FAS II reactions. As a key regulation point for fatty acid biosynthesis, ACCD6 carboxyltransferase can be a suitable target for new antimycobacterial agents. There is however no data concerning real function, regulation of *accD6* expression *in vivo* and the mechanism of acetyl-CoA carboxylation in fast and slow-growing mycobacteria.

By the use of directed mutagenesis and by generating a conditional mutants we demonstrated that *accD6* gene is essential for *M. tuberculosis*. Our genetic and proteomic studies have revealed that despite identical sequence and chromosomal organization, the expression of *accD6* gene is differently regulated in *Mycobacterium tuberculosis* H37Rv and non-pathogenic *Mycobacterium smegmatis* mc^2 155. We showed for the first time a definitive genetic proof that opposite to pathogenic Rv strain, *accD6* can be removed from genome of *M. smegmatis*. Deletion does not cause any changes in the structure, composition nor in the permeability of the mutant cell wall, however it induces changes in expression of other carboxyltransferases, crucial for mycolates biosynthesis.

The results indicate that ACCD6 carboxyltransferase from *M. tuberculosis* is essential for cell survival and can be concerned as a potential target for new tuberculostatics. This feature is specific only for pathogenic strain so *accD6* can be removed from the chromosome of *M. smegmatis*. Our work shows fundamental difference in acetyl-CoA carboxylation process between pathogenic and non-pathogenic strains of mycobacteria. It also suggests the existence of mycobacterial strains which carry out this process through alternative pathway, involving other carboxyltransferases. Finally we demonstrate that for some mycobacterial strains not all members of FASII operon are essential for the proper mycolates biosynthesis.

Keywords: Mycobacterium; mycolic acid; FAS, ACCase

Evaluation of photobactericidal effect of poly-L-lysine-chlorine p6 conjugate in *Pseudomonas aeruginosa* infected wounds

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Management of nosocomial infections caused by an opportunistic pathogen, *Pseudomonas aeruginosa* is a cause of concern due to its intrinsic resistance to drugs and the acquired antibiotic resistance prevalent in hospitalized environment. In this study, we report our investigations on use of poly-L-lysine-chlorine p6 conjugate (plcp6), a photosensitizing drug for inactivation of *P. aeruginosa* under in vitro conditions and in infected wounds of immunocompetent and immunocompromised (neutropenic) mice. Under in vitro conditions, 99.9% of bacteria were killed following photodynamic treatment (PDT) with 1.0 μM plcp6 and irradiation with red light (660 nm) at a dose of 25J/cm^2. PDT damaged cell envelop of bacteria. For treatment of infected wounds, plcp6 was topically applied and subsequently exposed to red light. ~90% of bacteria were killed when infected wounds were treated with 200μM plcp6 and irradiated with red light at a dose of 120 J/cm^2 in both immunocompetent and neutropenic mice. Further, while all the immunocompetent animals infected with bacteria subjected to different treatments survived, only PDT treated neutropenic mice survived beyond 48h post infection. In immunocompetent mice, PDT treated wounds healed faster compared to infected wounds treated with only plcp6 or not treated with either light or photosensitizer (Figure 1).These results demonstrate that photodynamic treatment using plcp6 is effective for both reducing the bacterial counts in wound as well as modulating wound healing. Details of these studies will be presented.

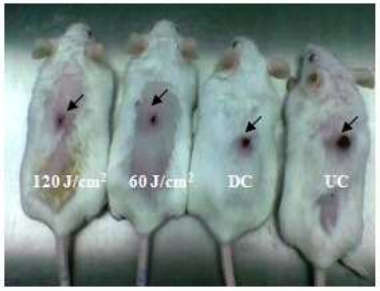

Figure 1: Photodynamic treatment of wounds using plcp6, in immunocompetent mice. Black arrow high-light wound in each experimental group on 14th day after photodynamic treatment. DC: Dark control; infected wounds treated with photosensitizer but not exposed to light, UC: Untreated control; infected wounds treated with neither photosensitizer nor light.

Keywords: Photodynamic treatment; infection; wound

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Experimental planning can help to optimize the selective photoinactivation of *Candida albicans* with Hypericin

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The genus Candida includes different species that have the potential to invade and colonize the human body. *Candida albicans* is the most common etiologic agent of skin, nail and mucous infections. The increasing resistance against antifungal drugs has renewed the search for alternative treatment procedures and antimicrobial photodynamic inactivation (PDI) is a propitious candidate. This technology is based on oxidative destruction of biological molecules by active oxygen species generated by photo-excited molecules (photosensitizers). Due to the large number of potential targets there is no cell resistance developed using this method. In PDI, the controlled or independent variables are: the sensitizer concentration, the incubation time of the cells with the drug solution, and the light dose administered to the cells containing the photosensitizer. The result or dependent variable is the survival index of the microorganisms and of the mammalian cells. The aim of this study was to use experimental planning in order to find the optimal experimental conditions, which could lead to selective inactivation of the fungi *C. albicans* with minimal damage to the host cells, what means to kill the microorganisms without significant level of damage to the host cells. An epithelial cell line was used as a model. The employed photosensitizer was Hypericin, a very active natural pigment found in plants of the genus Hypericum. The Hypericin used in this study was synthesized from emodin in Brazil. The visible light source setup was developed at the Institute of Physics – USP in Brazil and is constituted of a set of yellow LED (maximum at 590 nm). The human larynx tumor cells, HEp-2 (CCL-23) and *C. albicans* (ATCC 18804) were submitted to photodynamic treatment under the same conditions. Twenty different combinations of the independent variables were used. The results showed that the more significant factor for selectivity is the photosensitizer concentration. The obtained optimal conditions for selective inactivation were: incubation time of 150 seconds, 1 μg mL^{-1} Hypericin concentration and light dose of 2.9 J cm $^{-2}$. Hypericin accumulates very slowly in HEp-2 cells so that in this low incubation time, no significant cellular death occurs in the mammalian cells. Then, a careful control of the experimental parameters allows achieving a preferential phototoxicity of Hypericin against *Candida*. This approach can be very useful to search for the best conditions for PDI of *C. albicans* causing minimum damage to important constituents of host tissues, such as mucosas, skin and cavities. It is important to mention that our present results are valid for cells in suspensions. These optimal conditions found in this study needs to be tested in animal models. If these conditions show to be effective, a phototherapeutic protocol for clinical applications would be available to inactivate *C. albicans* in the oral mucosa in immune-compromised patients.

Keywords: *Candida albicans*, photoinactivation, hypericin

Fluorescent miltefosine (hexadecylphosphocholine) analogues as novel diagnostic tools to early monitor miltefosine-resistant *Leishmania* and differential ocular protozoal infection.

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Miltefosine (hexadecylphosphocholine, MT), initially developed as antitumoral drug has become nowadays the first the first successful oral drug against human visceral leishmaniasis, even against resistant isolates to organic pentavalent antimonial, until recently the golden standard treatment for this protozoal infection (1). The long half-life in vivo of miltefosine, the spurious access to the drug out of clinical control in India and the easy induction of resistance in vitro under drug pressure, foresees appearance of resistance a likely event in a near future. Previous evidence shows that MT resistance in *Leishmania* is almost exclusively due to the lack of function of its uptake system, an aminophospholipid flipase and its regulatory subunit (2). As such, appearance of resistance strains can be easily detected by incorporation of fluorescent analogues provided they were specifically recognized by this transporter and accumulated inside the parasite, as the parent drug.

To this end, we developed two classes of fluorescent miltefosine analogues differing in the nature of their respective fluorophor label. Selected surrogates of both groups were tested and successfully differentiated susceptible vs resistant *Leishmania* parasites. The first class of analogues, in which the emitting tag was a phenylpolyene, showed rapid photobleaching and required sophisticated detection devices (3) but in contrast to the 1st generation, that showed rapid photobleaching and required sophisticated detection devices (3). The 2nd generation, with a BODIPY emitting group, improved considerably photostability and were easily visualized in standard fluorescence microscopes.By means of fluorescent MT analogues, the measure of the intracellular concentration of the drug in *Leishmania* was estimated in the low micromolar range of concentrations. This value supports a multitarget lethal mechanism of MT and points to the extreme difficulty to develop resistance out of a faulty accumulation. Furthermore, reversible conjugation of MT to cell penetrating peptides abrogated initial resistance to the drug by opening an alternative entrance pathway independent of the canonical transporter. These conjugates also broaden the range of MT susceptible parasites, otherwise resistant by the lack or low expression of uptake sytems..

As MT was also incorporated by other parasitic Protozoa (4), we have also used these fluorescent analogues to facilitate *Acanthamoeba* detection and to selectively differentiate from fungi, both causative agents of ocular keratitis, paving the way for an in situ differential diagnosis of these two pathogens.

The potentiality of these analogues in ongoing clinical and academic applications will be discussed.

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Keywords miltefosine, *Leishmania* resistance, *Acanthamoeba*, fluorescent drug, early resistance diagnosis

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FtsQ division protein interactions as potential targets for new antibacterial drugs

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New antibiotics are urgently needed to treat the increasing number of life-threatening bacterial infections that are resistant to current therapies due to the widespread problem of bacterial resistance towards existing drugs. The paucity of effective drugs for the treatment of bacterial infections prompted the scientific community to think about novel strategies for discovering new classes of antibacterial agents, since, actually, most of the new drugs are merely variant of older overused antibiotics. Among the new putative targets, bacterial cell division is one essential process that is not yet targeted by clinically approved antibacterials and, moreover, most of the divisome components are characterized by essentiality and prokaryotic specificity. Bacterial cytokinesis is orchestrated by the divisome, a complex of proteins that co-ordinates and regulates the invagination of the cytoplasmic membrane, inward growth of the peptidoglycan layer and the outer membrane. To initiate cell division, the GTP-binding tubulin-like FtsZ protein forms an intracellular ring at the division site localized equidistant between the two cell poles, by FtsZ monomer self-assembly. The predominantly cytoplasmic proteins FtsA, ZipA and ZapA, which are the first to be recruited to the Z ring to form a complex with FtsZ. These proteins are responsible for tethering FtsZ to the membrane and stabilising the Z ring. These early recruits are followed by a group of single- or multipass membrane proteins that include bitopic (FtsQ, FtsL, FtsB, FtsN, FtsI) and polytopic (FtsK, FtsW) membrane proteins.

In these last 10 years, various natural or synthetic inhibitors against FtsZ protein or the FtsZ-ZipA complex or FtsA were identified. Although it is not known whether or not these compounds could have a medical application, these data confirm the hypothesis that these proteins represent an excellent antibacterial target. Other divisome components would be taken into account as potential targets for new antibiotics, and, amongst these, FtsQ, a highly conserved component of the divisome, that forms a complex with two other cell division proteins, FtsB and FtsL, necessary for linking the upstream division proteins, predominantly cytoplasmic, with the downstream predominantly periplasmic division proteins. The FtsQ location, external to the cytoplasm, allows to the inhibitors a more easily accession to this protein than intracellular proteins and removes the problematic issue of resistance development due to drug-efflux pumps. Furthermore, unlike FtsZ and FtsA, there are no identified human homologues to this protein, thereby increasing its potential as antibacterial target drugs. FtsQ is able to interact with various division proteins. Its domains involved in these interactions were identified by two-hybrid assays, co-immunoprecipitation experiments, and progressive deletions of the *ftsQ* gene. In addition, the selection and the study of FtsQ interaction-defective mutants constituted the basis for identify the FtsQ residues involved in the interaction with the other partner proteins and to investigate the biological significance of these interactions. The obtained results highlight that mutations in the POTRA domain strongly affect the functionality of FtsQ, assigning to this domain a prevalent role in the biological effects of this protein.

In order to evaluate the possibility to identify FtsQ, and, in particular its POTRA domain, as a potential target for new antibacterial drugs, we performed competition experiments where a protein fragment containing the POTRA region, was used as inhibitor of the bacterial growth. Although preliminary, our results suggest that new antibacterial agents inhibiting the POTRA domain would be explored. In particular, synthetic oligopeptides designed on the POTRA region would be used as antibacterial drugs. Moreover, since two separates sites for both FtsQ homodimerization and FtsI and FtsN interactions were identified, in order to maximize the inhibitory effect a cocktail of oligopetides derived from these two regions could be used to disrupt the bacterial division machinery.

Keywords divisome, protein-protein interaction, interaction-defective mutants

Genotypic identification and differentiation of Mycobacterium tuberculosis and related species by Multiplex Polymerase chain reaction (PCR) from clinical isolates of Kolkata, India.

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Historically tuberculosis is one of the oldest infection affecting human races. *Mycobacterium tuberculosis*, the causative agent of human TB is the commonest pathogen for pulmonary and extra pulmonary tuberculosis cases. But the other members of the *Mycobacterium tuberculosis* complex (MTC) or the nontubercular mycobacterium (NTM) produces similar diseases which cannot be differentiated from tuberculosis by clinical symptoms and signs. However, this differentiation is important as the chemotherapy varies widely according to the strain of infecting mycobacterium. The burden of morbidity and mortality of tuberculosis is rapidly growing worldwide, particularly with the forward march of HIV/AIDS epidemic. In laboratory strain identification of Mycobacterium remains a cumbersome, labor intensive and expensive procedure, which requires 3 to 12 weeks of time. The conventional methods of strain identification by primary isolation on culture, followed by observation of pigment production and series of biochemical tests lack proper standardization and precise diagnosis. Further, species identification is not possible in culture negative samples. This study aims to overcome these problems.

A multiplex Polymerase Chain Reaction (PCR) using 3 amplicons of 165,365, and 541 base pair target sequences was done with the clinical isolates of suspected Koch's patients. A total number of 165 clinical specimens (sputum, blood, body fluids etc.) of patients suffering from tuberculosis were included in the study .Strain identification was done both by conventional method and multiplex PCR. The results of the study show that this multiplex PCR is supposed to be less complicated, less time consuming and superior to the conventional methods of culture and biochemical tests. It is also applicable for culture negative samples where strain identification is not possible by conventional approach.

This technique may be of immense help , particularly in the third world country like India, where sophisticated molecular biological tools like LIPA, sequencing, DNA fingerprinting etc. are not readily available.

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High throughput assessment of the inhibition spectrum and potency of a liquid smoke condensate and selected components.

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Liquid smoke is used as a replacement for normal smoking of foods which has traditionally been used for preservation of food with respect to microbial and oxidative stability. To investigate the antimicrobial spectrum of a liquid smoke condensate we used a range of 44 indicator strains selected to comprise Gram-positive bacteria, Gram-negative bacteria and yeasts and moulds and to represent both spoilage organisms and pathogens. The liquid smoke condensate was initially tested at 1% v/v using a semi-automated spot-on-lawn method where it showed at broad inhibition spectrum comprising most Gram-positives, Gram-negatives and some yeasts and moulds.

Using a fully automated robotic system minimum inhibitory concentrations (MICs) were determined using the same indicator strain range. The liquid smoke condensate showed MICs between 0.026%and 0.93% for Gram-positive bacteria (average 0.22%), between 0.27% and 0.62% for Gram-negative bacteria (average 0.46%) and MICs between <0.026% and 0.95% for yeasts and moulds (average 0.52%). MICs were in general higher than the recommended dosage level (0.1-0.2%) meaning that as expected combinations with other antimicrobials and preserving processing techniques will most likely be necessary to have sufficient microbial stability at organoleptically acceptable levels. The carrier polysorbate 80 was tested against the same range of indicator strains and found to show no inhibition at a level of 5% and below. To assess the contribution of the acid component of the liquid smoke to antimicrobial activity, MICs were performed with acetic acid as well on the same indicator strains and some correlation was seen. However, the results demonstrated that the acetic acid component was not the only component contributing to the inhibitory activity. The BacLight staining kit combined with fluorescence microscopy was used to distinguish live and dead bacteria after exposure to different concentrations of liquid smoke. In general results correlated with MICs and the clear red staining as well as disintegration of cells indicated that liquid smoke has membrane disrupting capability.

Keywords Liquid smoke; antimicrobial; Gram-positive; Gram-negative; yeasts; moulds; MIC; dead/live; BacLight.

High-level expression of housefly cecropin A in *Escherichia coli* using a fusion protein

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Objective: The aim of this research was to investigate of the effectiveness of utilizing a molecular partner for high-level expression of *Musca domestica* cecropin in *Escherichia coli* and to identify the expressed products. **Methods :** The genomic sequence of *M.domestica* cecropin A gene (MC) and *M.domestica* ubiquitin (UBI) were searched from Genbank and amplified by reverse transcriptase polymerase chain reaction (RT-PCR). Two expression plasmids, pET32a–MC and pET32a–UBI–MC, were constructed and transformed into *E. coli* and were then induced using IPTG. The expression of the fusion proteins Trx–MC and Trx–UBI–MC was analyzed by SDS-PAGE. The Trx–MC fusion protein was verified by Western blot analysis. The bactericidal activity of the purified MC was quantitatively determined by MC against *E.coli* BL21(DE3). **Results:** The result showed that the fusion proteins were successively expressed in *E. coli* BL21 cells, a band at the expected position of 24 kDa representing the Trx–MC target protein was positively-stained , the band at 4 kDa representing the hydrolysis of mature MC protein was also observed at the expected position. The expression levels of Trx–UBI–MC were higher than Trx–MC in *E. coli*. MC exhibited antimicrobial activity. **Conclusions:** High-level expression of housefly cecropin A in *Escherichia coli* using a fusion protein, MC exhibited antimicrobial activity.

Key words: *Musca domestica*; Cecropin A; Molecular partner; Fusion expression; Antimicrobial activity

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High-throughput screening for altered persistence: finding new ways to combat *Pseudomonas aeruginosa*

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Infections caused by the gram-negative bacterium *Pseudomonas aeruginosa* are a major challenge. The combination of a low permeability of the outer membrane, efflux pumps and various resistance mechanisms limits the treatment options. In addition, the presence of persister cells makes it almost impossible to eliminate infections caused by this opportunistic pathogen. These persister cells are a small fraction of phenotypic variants that are extremely tolerant to prolonged treatment with high concentrations of antibiotics. If the antibiotic pressure drops, these persisters give rise to a population with the same antibiotic susceptibility as the original one. The molecular mechanism of this non-inheritable tolerance is still not unraveled. Here we describe a method that was used to identify mutants that affect persistence in *P. aeruginosa* and to screen a compound library for components that, in combination with ofloxacin, affect the survival of persister cells.

The screening method is based on differential survival after prolonged treatment with an antibiotic compared to a control treatment. Stationary phase cultures are treated with the fluoroquinolone ofloxacin. This antibiotic is lethal to non-growing cells in the stationary phase so that only persister cells survive. After this treatment, the cells are diluted in growth medium and incubated in an automated optical density (OD) plate reader to generate growth curves of the surviving cells. The number of surviving persister cells is directly related to the lag phase of the growth curve. The phenotype is confirmed independently using plate counts.

A library of 5000 *P. aeruginosa* PA14 mutants, constructed by random insertion of the pTn*Mod*-OGm plasposon, was screened for persistence mutants. Nine mutants consistently displaying an altered persister fraction compared to the wild type were selected. Four mutants showed a decreased persister fraction while five mutants exhibited a higher number of persisters. In addition, by screening a compound library, we discovered compounds that, when co-administered with ofloxacin, decrease the survival of persister cells. A further analysis of these compounds may in the future lead to novel therapies to combat chronic infections.

Keywords persistence; *Pseudomonas aeruginosa*; high-throughput screening

How cephalosporins cross the bacterial outer membrane and what have Omp's got to do with it. A combined biophysical and biological study.

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Multidrug resistance (MDR) is frequently reported in Gram-negative bacteria. These bacteria have a complex cell envelope, comprising a cytoplasmic membrane and an outer membrane containing several general diffusion channel proteins, porins. These pore-forming proteins are responsible for the influx of different families of antibiotics, specifically including cephalosporins. Recent clinical data indicate that bacterial adaptation to limit drug influx is a multifaceted problem, with several different cellular strategies of porin modification reducing drug susceptibility. This phenomenon has been described as one of the main causes of appearance and dissemination of antibiotic resistance, contributing to the emergence of pan-resistant strains. Studies carried out with clinical isolates show that cephalosporins, which are among the most frequently prescribed antimicrobials today, see their activity strongly affected by bacterial modulation of the outer membrane barrier.

Two major porins, OmpF and OmpC, are key players in antibiotic uptake in *Escherichia (E.) coli* strains and similar proteins have been described in other species of clinical importance. We focus on biological and biophysical characterization of antibiotic permeation using a systematic and multidisciplinary molecular approach that can help improve our understanding of the parameters that govern antibiotic translocation through porin channels.

Analytical considerations indicate that affinity of small molecules to the interior of diffusion channels enhances their flux. We have measured the interaction strengths of ceftriaxone, cefpirome and ceftazidime with the two most abundant outer membrane porins of *E. coli*, OmpF and OmpC in model membranes. Affinity constants were obtained by measuring the static quenching of inherent tryptophan fluorescence in the porins in the presence of the antibiotics. Through an empirical inner filter effect correction we have succeeded in measuring the chemical interaction of these strongly absorbing antibiotics, and obtained a satisfactory agreement with prior electrophysiology measurements The interaction of all three antibiotics is smaller for OmpC than OmpF, and in the case of each porin the interaction strength series ceftriaxone > cefpirome > ceftazidime is maintained. This correlates well with the antibiotics' biological activity and accumulation in the presence and absence of these porins.

Biological studies were also performed in a series of porin-deficient *E. coli* strains (OmpF, OmpC, lamB). This combined study of the interactions at a single molecular level and *in vivo* can provide new insights for a better understanding of the antibiotic translocation.

Keywords Cephalosporins, model membranes

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Identification of *Mycobacterium Chelonae* isolates in the sputum sample from Patient with breast cancer in Tehran

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Background. Mycobacterium chelonae is nontuberculous mycobacteria (NTM), a grouping that encompasses all mycobacteria outside of the Mycobacterium tuberculosis complex. M chelonae causes various clinical syndromes, including lung disease, local cutaneous disease, osteomyelitis, joint infections, and ocular disease. With the exception of lung disease, these syndromes commonly develop after trauma. M. chelonae is a rare cause of isolated lymphadenitis. Endocarditis has also been documented. Disseminated disease, usually with disseminated skin and soft tissue lesions, occurs almost exclusively in the setting of immunosuppression, especially AIDS. Esophageal disorders may place patients at increased risk for pulmonary disease due to rapidly growing mycobacteria. Surgical-site infections due to M chelonae are well documented, especially in association with cardiothoracic surgery and augmentation mammoplasty. Alternative practices such as mesotherapy have been associated with skin infections. No human-to-human transmission has been documented.

Material and Method. Sputum was collected from suspected non-tuberculosis patient case had proven registration of clinical diagnostic examination. This isolate was cultured on Lowenstein Jensen solid medium and grown colonies after 7 days. All identification testing and drug susceptibility testing was done accordance CDC standard method. PCR amplification with several primers for identifying complex tuberculosis from Chelonae was performed.

Result. Acid fast staining, uramin and culture tests has been positive. Tuberculin, PCR amplifications has been negative. Growth on 2- thiphene carboxylic acid medium was positive in Lowenstein Jensen and colonies of bacteria was non- photochromogen. Catalase test in two temperatures has been positive and niacin- nitrate tests was identified negative.

Conclusion. In this study, this isolates was from mycobacterium chelonae complex that has been isolated from patient with breast cancer and virulence of M. chelonae is in the human with other problem consist of cancer, autoimmune, Allergy, osteomyelitis and etc.

Interaction of antimicrobial lipopeptides with bilayer lipid membranes of different dipole potential

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Membrane dipole potential originates from dipole moments of lipid molecules and adjacent water dipoles in interfacial region. The dipole potential regulates numerous biological processes, including membrane ion permeability, membrane-bound protein folding, protein insertion, oligomerization, and functioning. We have studied an effect of bilayer dipole potential on channel-forming activity of antifungal cyclic lipodepsipeptides from *Pseudomonas syringae*, syringomycin E (SRE) and syringopeptin 22A (SP), as well as of antimicrobial cyclic heptapeptide from *Bacillus subtilis*, surfactin (SA). "Solvent-free" planar phospholipid bilayers were prepared using monolayer-opposition technique. The membrane dipole potential was modified by adding phloretin or styryl dye RH 421 to the bilayer bathing solutions.

We have found that an increase of the bilayer dipole potential leads to significant increase of the channel-forming activity of SA, and significant decreasing of SRE- and SP-activities. These alterations were determined by several factors:

1. partitioning of lipopeptide molecules between bilayer and aqueous solution,
2. modification of a multilevel channel conductance, i.e. a change of the number of monomers in conducting surfactin oligomers or cooperatively functioning elementary channels in syringomycin clusters;
3. voltage-gating properties, related to translocations of lipid and syringomycin E or syringopeptin 22A dipoles in the process of channel formation.

We do not also eliminate contribution of the chemical component, related to conformational changes during pore formation, in modulation of lipopeptide activity in membranes of different dipole potential.

In the case of raft-containing membranes the channel-forming activity of syringomycin E was also determined by partitioning of dipole modifiers between ordered and disordered membrane regions.

The presented data allow us to propose that the dipole potential of target cell membranes greatly affects antimicrobial activity of the studied lipopeptides.

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Keywords bilayer lipid membranes, antimicrobial lipopeptides, membrane dipole potential, ion channels

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Microbiological evaluation in Chestnut-of-Brazil after ionizing process

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The chestnut-of-brazil is a product found in tropical rainforest that the large plant size is between 30 to 50 meters high that it makes difficult harvesting of the fruits (called hedgehog). For this reason, the collect of the hedgehog deserves special attention, since it is an important source of nuts contamination, due to the contact with the ground floor for a period that can range from a few days to 4 months. During this stage, it is observed a process of decomposition of the organic substrate attached externally to the hull with consequent proliferation of microorganisms. The presence of bacteria from the coliform group, mainly thermotolerants coliforms (fecal), although not constitute a direct biological hazard, act as an indicator of environmental contamination. This is caused by the improper handling of raw material and final product as well as poor hygiene practices. Potential pathogenic bacteria as well as *Salmonella* may contaminate the nuts during all the supply chain. Foods treated with ionizing radiation results in a large reduction in microbial contamination. The aim of this study is to verify the microbiological aspects after applying the irradiation process using ^{60}Co source Gammacell 220 (A.E.C. Ltda) at doses of 0, 1.5 and 3kGy.

Keywords: food irradiation, microbiology; Chestnut-of-Brazil; *Salmonella* ssp.

Microbiological hazard identification and exposure assessment for *Klebsiella pneumoniae* by consumption of lettuce salad in restaurants in southeastern Brazil

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The microbiological quality of the lettuce distributed in restaurants at Viçosa city, southeastern Brazil, was evaluated using a HACCP based approach. This includes information on pathogens of potential importance and impact of process steps and post-process on concentration and prevalence. The population is 74,171 habitants and the Municipal Health Department reported that there are 46 restaurants. Three representative restaurants that serve about 2,000 meals per day and three small farmers daily supply those restaurants with vegetables were selected for this research. We investigated the habits of these consumers and found out that children and adults between 10-70 years old, consume small (8.0 g), medium (20.0 g) and large portions (30.0 g), in a single meal from three to seven times per week. The lettuce salad is daily consumed by over 95 % these clients.Eighty one lettuce samples from farm (n = 27), from delivery to restaurant (n = 27) and the ready to eat (n = 27) were collected. The microbiological analysis showed *Escherichia coli*, *Cronobacter sakazakii* and *Klebisiella pneumoniae* as potentially harmful microorganisms present in lettuce, including the lettuce salad served in the restaurants. *K. pneumoniae* was detected in 81.5% of the ready to eat lettuce samples, in 3.5 log cfu·g^{-1} at restaurant A, 2.44 log cfu·g^{-1} at restaurant B, and 3.43·log cfu·g^{-1} at restaurant C, denoting unsatisfactory hygiene conditions during food handling. The growth of *K. pneumoniae* on fresh-cut lettuce was modelled in order to investigate microbial safety distribution of this vegetable on restaurants. We analyzed the effects of incubation temperatures, between 5 ºC and 30 °C, on bacterial growth. These data were fitted using primary Baranyi model and the curves showed a high correlation coefficient (R^2>0.95), except at 5 ºC. In storage at 5 ºC, 10 °C, 20 °C and 30 °C the duration of the lag phases were predicted at 49 h, 22 h, 1.74 h and 1.13 h and the specific rates of maximum growth at 0.022·h^{-1}, 0.070·h^{-1}, 0.199·h^{-1} and 0.540·h^{-1}, respectively. The growth rates of inoculated *K. pneumoniae* on lettuce were described using Ratkowsky's square root model. The statistical parameters that validated the model were bias factor (0.9959) and accuracy factor (1.0379).

According to the predicted values the adaptation time decreased when the temperature increased. The consumer that to eat 30 g of lettuce in one of the restaurants will probably be ingest more than 5 log cfu·g^{-1} of *K. pneumoniae* cells after 3 h at 20 ºC.

Keywords*:* lettuce; *Klebsiella pneumoniae*; bacterial growth

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New Approaches for bacterial detection in technical fluids

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This paper presents first results of a new strategy for the detection of organic/biological contamination using the Surface Plasmon Resonance sensor (SPR) technology. SPR sensors have been commonly applied for the detection of proteins, bacteria and chemical substance. However, only specific interactions can be detected so far that rely on specific and sensor immobilized receptor molecules. It is of importance to overcome this limitation. The here presented new approach will focus on a distinguished mode of action. The main principle of the presented sensor operates without a flow cell and allows non-specific detection of biological content in a fluid domain, because the reaction kinetics are not being taken into account. Furthermore, there is the probability to disclaim the use of an organic matrix, such as the common dextran or fibrin matrices. Besides, one of the main advantages of the sensor is the usability in a multitude of liquids such as emulsions or deeply contaminated ones, providing a broad applicability of the sensor system.

Keywords surface plasmon resonance, detection, fluid, surface modification

Novel Antibacterial Agents Targeting WalK/WalR, Two-Component Signal Transduction System, Essential for Cell Growth of *Staphylococcus aureus*

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Practically ubiquitous among bacteria, two-component signal transduction systems (TCSs) are coupled sensor histidine kinase (HK) and response regulator (RR) pairs allowing cells to rapidly adapt their genetic expression to environmental changes. These TCSs achieve this by controlling functions ranging from virulence, antimicrobial resistance, and biofilm formation to quorum sensing and cell growth(1). The YycG(HK)/YycF(RR) TCS, recently renamed WalK/WalR to reflect its function, is highly conserved and specific to low G+C Gram-positive bacteria, such as *Bacillus subtilis*, *Enterococcus faecalis, Streptococcus pneumoniae,* and *Staphylococcus aureus,* where it acts as a master regulatory system for cell wall metabolism and is essential for cell viability. As such, the WalK/WalR system constitutes an attractive target for the development of novel antimicrobial compounds, since inhibitors would be expected to have a bactericidal effect on a broad range of major Gram-positive pathogens.

With the aim of developing a new class of antibacterial agents, in this paper we introduce two drug discovery systems to isolate the inhibitors of the WalK/WalR system: 1) differential growth assay with a temperature sensitive *walR* mutant; 2) a high-throughput genetic system for targeting the homodimerization (HD system) of WalR. By using such screening systems, we discovered two new antibacterial agents (walkmycin and walrycin) targeting WalK and WalR, respectively, which also showed bactericidal effects on MRSA and VRE (2-4).

Keywords two-component signal transduction;WalK; WalR; MRSA; VRE; histidine kinase inhibitor,

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Photosensitization as human and environmentally friendly antimicrobial tool

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The discovery of antibiotics raised the belief that human being has found powerful tool to control pathogens and infectious diseases. Unfortunately, after seventy years of hard work we must state, that the fight against microbes is still continuing and remains as one of the permanent challenges.

Despite tremendous progress in food microbiology, the number of reported food-borne diseases continues to rise. Health experts estimate that every year food-borne illnesses in USA cost 5-6 billion US dollars in direct medical expenses and lost productivity. Infections with the bacteria Salmonella alone account for 2.5 billion dollars yearly. Thus, food-borne diseases are extremely costly(CDC, 2004).

Photosensitization is a treatment involving the interaction of the two non-toxic factors, photosensitizer (can be chlorophyll, plant pigment hypericin etc.) and visible light, which in the presence of oxygen results in the selective destruction of the target cell. Different microorganisms, such as multidrug-resistant bacteria, yeasts, microfungi and viruses are susceptible to this treatment. As a rule, the photosensitizer accumulates in the cell wall. After irradiation by visible light, reactive oxygen species induce rapid disruption of the cell wall. Reactive ROS interacts with unsaturated fatty acids, amino acid residues, such as cystein, histidine, tryptophan, nucleic acid bases of DNA, particularly guanine and thymidine. Breaks in both single- and double-stranded DNA have been detected in both Gram (+) and Gram (-) bacteria after photosensitization.

The accumulation of photosensitizer in the cell strongly depends on the physiological state of bacteria: in the exponential growth phase bacteria exhibit better accumulation of the photosensitizer than corresponding cells in the lag phase. Moreover, spores which are usually more resistant to different treatments are susceptible to photosensitization. Other physiological condition- microbial biofilms being more resistant to any antibacterial treatment are susceptible to photosensitization.

High antimicrobial efficiency of photosensitization was used to inactivate harmful and pathogenic microorganisms on the surface of different foods, to sterilize various surfaces, including packaging, industrial premises and instruments in environmental friendly, non- thermal and not mutagenic way.

Therefore, a photosensitization phenomenon might open a new avenue for the development of non-thermal, effective and ecologically friendly antimicrobial technology.

Rapid Diagnosis of Neonatal Bacteremia Using Polymerase Chain Reaction

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Infections in the neonate are the most important causes of mortality and hospitalizations in the neonatal practice. In the present study, 180 neonates with criteria for probable sepsis admitted to neonatal intensive care unit in Ahmad Maher Teaching hospital were investigated for evidence of sepsis. The investigation protocol included sepsis screen, blood culture and molecular analysis by polymerase chain reaction (PCR) for bacterial DNA component encoding 16S rRNA in all cases. We compared the results of PCR with blood culture and other markers of sepsis screen. Blood cultures were positive in 80 (44.4%) cases with sensitivity of 91.9%. PCR was positive in 87(48.3%) cases (49 of them are with positive blood culture, CRP and buffy coat test). The sensitivity of PCR was 100% and specificity was 93%. The negative predictive value was 100%. The study concluded that although blood culture is a reliable method for diagnosis of neonatal sepsis, the universal bacterial primer PCR is a useful test and superior to blood culture for early diagnosis of sepsis in neonates. Consequently unnecessary treatment with antibiotics could be avoided.

Keywords: neonatal sepsis; bacteria; PCR

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Rapid, Broad-Spectrum Antibiotic Resistance Marker Detection Using High-Density Microarray.

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Background

The rising prevalence of multidrug resistant pathogens both in nosocomial as well as in community-acquired infections is becoming a major challenge for successful treatment of bacterial infections. According to a number of recent studies the situation is especially alarming in military medical treatment facilities, where exceptionally high proportion (>80%) of infections caused by multidrug resistant bacterial strains with resistance to >3 different classes of drugs was observed. One of the reasons leading to this situation is lack of quick drug resistance testing methods and reliance on empirical therapeutic approaches. Here we present a prototype Antimicrobial Resistance Determinant Microarray (ARDM) capable of rapid detection of a broad range of antimicrobial resistance determinants.

Methods

The Combimatrix, ElectraSense technology based ARDM microarray, containing 2240 DNA probes, that enables detection of 278 antibiotic resistance genes covering 12 classes of antibiotics, was designed and manufactured. The validation of this platform was conducted using 28 clinical isolates of *Acinetobacter baumanni*, *Escherichia coli*, and *Klebsiella pneumoniae* with known antibiograms.

Results

The microarray was able to detect antimicrobial resistance determinants belonging to 8 classes of antibiotics: β-lactam, aminoglycosides, macrolides, sulfonamides, diaminopyrimidines, tetracyclines, glycopeptides and quinolones.

The results of microarray analysis agreed in general with the results of culture based testing (E-test) although a number of false negatives were observed.

Conclusions

ARDM has a potential of becoming a valuable method of antimicrobial resistance determination especially in the settings where rapid diagnosis is essential and culture based testing is not practical.

Work is in progress on addressing the sensitivity issues and including additional resistance markers as well as probes enabling the identification of major groups of pathogens concomitantly with antimicrobial resistance profiling.

Keywords: Drug-resistance markers; detection; broad-spectrum microarray; ARDM.

Real-time monitoring of biofilm metabolic activity during antimicrobial treatment

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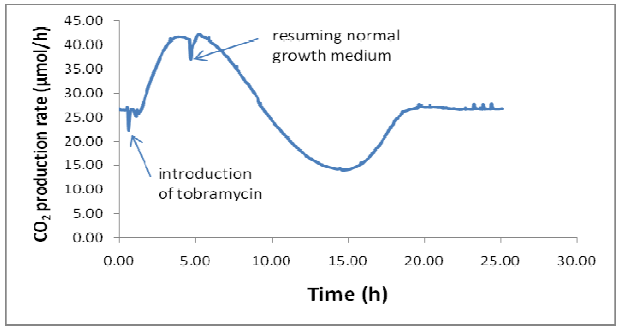
Several pathogens associated with chronic infections are capable of biofilm formation, where the lack of oxygen penetration (proposed to lead to slower growth) rather than reduced antimicrobial penetration into the biofilm is often cited as a reason for diminished antimicrobial killing efficiency.

The aim of this research was to monitor the metabolic response of mature *Pseudomonas aeruginosa* PA01 biofilms to NaClO and repeated tobramycin exposure in real-time, under aerobic and anaerobic conditions and to enumerate the number of viable cells yielded to the planktonic stage. A carbon dioxide evolution measurement system (CEMS) was used as a continuous flow biofilm reactor where the biofilm is formed inside a silicone tube and the gaseous CO_2 that permeates through the silicone is measured continuously with a CO_2 analyzer. The CEMS furthermore allows the measurement of metabolic activity at different temperatures and oxygen concentrations (e.g. by using anaerobic growth medium and changing the sweep gas to N_2). A typical response to antimicrobial treatment can be seen in the figure below. In this case the biofilm (grown at 37 °C) was treated with 50 mg/L tobramycin (at t = 0.5 h, 10 mg/L is the maximum therapeutic serum concentration) for a duration of 4 hours after which the supply of normal growth medium was resumed. The metabolism returned to pre-treatment steady state values within 24 hours. In further experiments, replicates of single biofilms were repeatedly exposed (3 h at a time) to 100 mg/L tobramycin on subsequent days resulting in almost identical transient increases in metabolism followed by recovery. Biofilm metabolism was reduced to a greater extent by tobramycin treatment at 37 °C as when compared to treatment at 27 °C where metabolism hardly fell below normal steady state values. Under anaerobic conditions, the biofilms reached higher steady state CO_2 production values but recovery was significantly retarded after tobramycin exposure when compared to aerobic conditions when citrate was supplied as the sole carbon source. When the biofilm was treated with ~100 mg/L NaClO for one hour, the metabolism dropped to 8% of pre-treatment steady state values and remained at that level for almost 20 hours. Pre-treatment values were regained 40 hours after the NaClO exposure. Cell numbers in the effluent dropped from ~10^6 CFU/ml to ~10^4 CFU/ml during the first hour after treatment with the ~100 mg/L NaClO and recovered to ~10^5 CFU/ml 3 hours after treatment.

Because of the non-invasive nature of this approach, it was possible (i) to track whole biofilm metabolic response in real-time during repeated antimicrobial exposures, (ii) to relate metabolic response with viable cell yield to the effluent, and (iii) to monitor the recovery of biofilm metabolism after the cessation of the antimicrobial treatment. The results showed that the *P. aeruginosa* PA01 biofilm response to tobramycin treatment differed with temperature and oxygen concentration and that slower growth due to lack of oxygen does not appear to be the only factor in tobramycin susceptibility.



Keywords biofilm; tobramycin, anaerobic

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Restoring β-lactam sensitivity of MRSA: Examination of the mechanism underlying combinatorial treatment with phenothiazine derivates

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Thioridazine is a phenothiazine derivate, which is known to resensitize methicillin resistant *S. aureus* (MRSA) to β-lactam antibiotics *in vitro* [1]. In this study we examine the molecular basis of the reversal effect.

S. aureus is a global cause of skin infections and invasive diseases, the treatment of which has been complicated by the emergence of clonal lineages displaying resistance to the most commonly used classes of antibiotics. The MRSA strains have acquired the *mecA* gene encoding a low-affinity penicillin-binding protein (PBP2a) that confers resistance to a broad spectrum of β-lactam antibiotics. As a consequence of the rapid development of multi-drug resistant bacteria and the time-consuming process of developing new drugs we have set out to explore the possibility of restoring the sensitivity of MRSA strains to the preferred class of antibiotics, the β-lactams, using phenothiazine derivates. Previously, we have demonstrated that thioridazine prevents oxacillin-induced transcription of *mecA* in the hospital-associated MRSA isolate ATCC 33591 [2]. However, due to strain-to-strain variation we are aware that this observation cannot fully explain how thioridazine reverses oxacillin resistance. Thus, in order to gain a deeper insight into the mode of action we have picked out a set of genes encoding proteins that are involved in cell wall maintenance and β-lactam resistance as well as a group of virulence genes and examined how the combinatorial treatment of thioridazine and the β-lactam dicloxacillin affects the mRNA levels. Similar to the effect on *mecA* expression, we show that thioridazine counteracts the induction of several VraSR-regulated genes in the presence of dicloxacillin. The two-component system VraSR is known to sense perturbations of cell wall synthesis and is of major importance to high-level β-lactam resistance as well as vancomycin resistance in *S. aureus* [3]. Contrary to the strong effect on VraSR-regulated genes, thioridazine and/or dicloxacillin only showed minor or no influence on expression of the virulence genes examined here. Interestingly, we saw that thioridazine gives rise to different transcriptional responses depending on the concentration used, suggesting that it affects several regulatory pathways in *S. aureus* in a concentration-dependent manner. Altogether, our results indicate that reversal of β-lactam resistance in MRSA is a consequence of additional effects of thioridazine on cell wall synthesis besides its influence on *mecA* expression. Studies are ongoing in our group to determine the exact role of the VraSR two-component system during reversal of resistance by thioridazine.

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Keywords resistance reversal; MRSA; phenothiazines; cell wall maintenance; two-component system VraSR

Screening the SIDR Natural Product Library for inhibitors of a novel DEAD box protein ATPase demonstrating substrate specificity to bacterial 23S rRNA

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A novel target protein for antimicrobial compounds has been developed at the University of Dundee. DEAD box protein (DbpA) from *E.coli* exhibits RNA-dependent ATPase activity with substrate specificity for bacterial 23S ribosomal RNA. Eukaryotic RNA does not stimulate the hydrolysis of ATP from DbpA. Therefore inhibitors of DbpA would be selective antibacterial agents. 4416 extracts from the SIDR Natural product library were screened for DbpA inhibition using a high throughput microplate ATP hydrolysis assay. Concentration response studies were carried out on the 80 most active extracts. Assays containing BSA were also conducted to identify non-specific interferences and indicate possible false positives. Methodology and experimental data will be presented and discussed.

Keywords DbpA; Inhibitor; ATP hydrolysis assay; antibacterial; natural product.

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Study of the interaction of an antimicrobial protein (*St*Asp-PSI) with phospholipid bilayer membranes

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Plant-specific insert domain (PSI) is a region of approximately 100 amino acid residues present in most plant aspartic protease (AP) precursors. PSI is not a true saposin domain; it is the exchange of the N- and C-terminal portions of a saposin-like domain. Hence, PSI is called a swaposin domain. Previously, we have reported the cloning, heterologous expression and purification of PSI from *St*Asp 1 (*Solanum tuberosum* aspartic protease 1), called *St*Asp-PSI. Additionally, we demonstrated that, like proteins of the SAPLIP family, *St*Asp-PSI is cytotoxic to human pathogens in a dose-dependent manner, but this effect was not observed on human red blood cells at all concentrations assayed. The *St*Asp-PSI ability to kill microbial pathogens is dependent on the direct interaction of the protein with the microbial cell membrane, leading to increased permeability and lysis. However, the mechanism by which *St*Asp-PSI interacts with the plasma membrane is not well understood. The aim of this work was to analyze the properties of *St*Asp-PSI-lipid interactions. The results obtained show that *St*Asp-PSI was able to cause vesicles surface destabilization and leakage in a dose-dependent manner, in vesicle composed by EPC:EPA (5:4) and EPC:EPG (5:4). A decrease in the *St*Asp-PSI leakage ability was detected when the vesicles were composed by zwitterionic lipids (EPC; EPC:TPE (5:2)) or a lipid extract from bovine liver. Additionally, the presence of cholesterol in the vesicle composition decreased the ability of *St*Asp-PSI to cause leakage in a dose-dependent manner. In order to analyze *St*Asp-PSI conformational changes, circular dichroism (CD) assays were performed. Data obtained show that *St*Asp-PSI helical conformations was reduced after incubation of this protein with EPC:EPA (5:2), EPC:EPG (5:2), and EPC:Chol (5:2) vesicles by 50, 70 and 20 %, respectively. Results obtained from differential scanning calorimetry method (DSC) show that *St*Asp-PSI was able to perturb anionic bilayers (composed by anionic dimyristoylphosphatidyl glycerol, DMPG and anionic dimyristoylphosphatidyl serine, DMPS). There was a lower effect of *St*Asp-PSI on zwitterionic bilayers (dimyristoylphosphatidylcholine, DMPC). Infrared spectroscopy (IR) spectra results show that minor changes were detected in the *St*Asp-PSI Amide I´ region, in the presence of anionic or neutral membranes. Therefore, the results obtained in this work suggest that: a- the content of higher amounts of anionic lipids in plasma membrane of prokaryotic cells, b- the decrease observed in the *St*Asp-PSI vesicle leakage ability in presence of cholesterol, and c- the fact that at lower amounts of *St*Asp-PSI vesicle leakage is only detected in negative vesicles, could explain the differences observed in the ability of *St*Asp-PSI to kill human bacterial pathogens (*E. coli*, *S. aureus*, and *B. cereus*) but not human cells (lymphocytes and erythrocytes).

Keywords: plant-specific insert, antimicrobial activity.

Study on The Effect of Plasma Pretreatment on the Antimicrobial Efficacy of Neemleaf Extract Processed Cotton Fabric

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It has long been recognized that the microorganisms can thrive on textile materials. Cotton, which is one of the most commonly used fabrics to make surgical garments and drapes is especially more prone to attack because of its hydrophilic structure which retains water, nutrients etc. The use of surgical garments has evolved as a standard practice and their primary purpose is to protect the health care team in the surgical zone from microbial invasion and fluid borne pathogens. Giving due importance to this property, the objective of the present investigation is to improve the antimicrobial efficacy of cotton fabric treated with neem leaves using plasma pretreatment. Extensive and exhaustive research on neem tree by various groups all through has indicated that every part of neem tree (bark, leaves, seed etc.,) contains a plethora of triterpeniods like azadirachtin, nimbin, nimbidin, nimbolide, gedunin etc., that are bioactive.

In the present study, 20 count 100% pure cotton fabric used in the surgical zone has been treated using RF plasma with air, oxygen and argon as process gas to improve its hydrophilicity prior to the application of methanolic extract of neem leaves.

A detailed investigation has been carried out to understand the role of plasma in modifying the fabric surface and in turn the improvement of the antimicrobial efficacy when treated with neem leaf extract. During RF oxygen plasma treatment the process parameters such as RF power has been fixed and the pressure, electrode gap and exposure time have been varied. However, while treating the fabric with the RF air plasma the gas pressure has been fixed and the parameters namely RF power, electrode gap and exposure time have been varied. During RF argon plasma treatment all the four process parameters have been varied. The hydrophilicity of all these samples has been assessed using standard tests and the process parameters have been optimized for maximum hydrophilicity. The fabric samples that exhibited maximum hydrophilicity in these processes have been subjected to chemical and ATR-FTIR analysis to study the reaction mechanism that has occurred during plasma treatment. The studies reveal that the primary alcohol present in the cellulose on the surface of the fabric has been oxidized to carbonyl and carboxyl groups.

The SEM analysis has been carried out on the samples to understand the morphological changes that occurred during plasma treatment. The effect of plasma treatment on the mean pore diameter of the fabric matrix has been analyzed using dynamic wicking test and air permeability test. The results show that the wickability of the fabric has been improved due to the increase in mean pore diameter of the fabric matrix.

Thus, it has been confirmed that the plasma treatment improves the hydrophilicity of the cotton fabric by inducing both chemical and physical change on the fabric surface due to which, its uptake is improved when treated with neem leaf extract. This is reflected in its antimicrobial efficacy when tested using standard assays.

An attempt has also been made to understand the improvement in durability of the finish by analyzing FTIR spectra of the samples. The analysis confirms the formation of ether and ester that is responsible for increased durability.

Keywords: Cotton fabric, Plasma pretreatment, Hydrophilicity, Antimicrobial finish, Methanolic extract of neem leaves, Chemical analysis, ATR-FTIR spectrum, , SEM micrographs,

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Tetracycline and trimethoprim-sulfamethoxazole at clinical laboratory: can it help to characterize *Staphylococcus aureus* carrying different SCC*mec* types?

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Methicillin resistant *Staphylococcus aureus* (MRSA) is an important pathogen which presents a penicillin binding protein 2a (PBP2a) encoded by the *mecA* gene that is located in a mobile genetic element called staphylococcal cassette chromosome (SCC*mec*). The most frequent SCC*mec* types found are I, II and III in hospital isolates and IV associated with community-acquired. In Brazil, isolates carrying types III and IV have been found in hospitals. We analyzed 140 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from inpatients in four hospitals in Rio de Janeiro city, between 2004 and 2007, in order to assess the antimicrobial susceptibility and the SCC*mec* types. The susceptibility to 13 antimicrobials was determined by the disk diffusion method. The Minimum Inhibitory Concentration (MIC) determination for TMP/STX and tetracycline was also performed by the broth microdilution method. Type III isolates (63 strains) were more resistant to ciprofloxacin, clindamycin, cloramphenicol, erythromycin, gentamicin and rifampin than type IV ones (65) ($p < 0.05$). Moreover, they were susceptible to tetracycline (100%) and to TMP/STX (98%), but all type III isolates presented resistance to both antibiotics. Disk diffusion and MIC tests were 100% compatible. MRSA isolates carrying SCC*mec*IV presented MIC_{90} to TMP/STX and tetracycline, $\leq$0.125/2.375 and $\leq$0.5, respectively. In opposite, type III isolates showed MIC_{90} $\geq$32/608µg/mL to TMP/STX and 32µg/mL to tetracycline. We can suppose that in regions where the types III and IV of SCC*mec* are prevalent, the detection of specific resistance phenotypes could help to distinguish them, mainly when there were no technical conditions to typing the isolates.

Keywords: MRSA, *SCCmec*, trimethoprim/sulfamethoxazole, tetracycline

The Minimum Inhibitory Concentration: a gold or tarnished standard?

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According to current wisdom, the Minimum inhibitory concentration (MIC) is "the 'gold standard' for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing." (Andrews 2001). And, "like all standardised procedures the method must be adhered to and may not be adapted by the user." But can you create if you conform? Stripping away the rigid methodology reveals a highly tarnished, if not brass, standard and a system incapable of providing the serious researcher with quality information. If the 'Gold standard' is an inappropriate method for research (as opposed to conducting a test) then systems based on it must also be flawed: for example the current concept of antimicrobial synergy, used within pharma and other industries, for the analysis of combined effects can be shown to suffer from a deficient standardised MIC test.

In this talk a discussion of antimicrobial analysis begun in Unilever, developed further in Nestlé and further refined in academia will be presented.

Keywords minimum inhibitory concentration, susceptibility, synergy

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Trypanocidal action of eupomatenoid-5 on epimastigote forms of *Trypanosoma cruzi*

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The protozoan *Trypanosoma cruzi* is the etiological agent of Chagas disease, an infection that affects millions of people but unfortunately has no satisfactory treatment. The search for new drugs able to cure or even avoid the disease progress is ongoing, and natural compounds are a good alternative for the treatment of this infection. Recently, eupomatenoid-5 isolated from leaves of *Piper regnellii* var. pallescens was described to have an antiproliferative effect on *T. cruzi*. To improve this study we performed the hemolytic assay to discard the human toxicity of eupomatenoid-5 and as a way to study the mechanism of action of eupomatenoid-5 we analyzed the formation of autophagyc vacuoles on epimastigotes of *T. cruzi*. In face of that, eupomatenoid-5 in a range of 10 – 500 µg/mL was incubated for 3 h at 37 °C with a suspension of 3% human red blood cells. Next, the absorbance of the supernatant was obtained by spectrophotometric at 550 nm. To assess the formation of autophagic vacuoles epimastigotes of *T. cruzi* were pretreated with eupomatenoid-5 (IC50 = 7 µg/mL and IC90 = 15 µg/mL) for 96 h, followed by monodancyl cadaverine staining and analyzed by fluorescence microscope. The results showed eupomatenoid-5 has no toxicity on human erythrocytes in all concentrations tested. On the other hand, eupomatenoid-5 showed to be responsible for beginning a process of autophagy on epimastigotes of *T. cruzi*. These data direct our goals to further mechanistics studies involving the metabolic balance of *T. cruzi* since autophagy is a process by which eukaryotic cells degrade and recycle macromolecules and organelles making this process crucial to the maintenance of metabolic balance. Additionaly, eupomatenoid-5 seems to be a good tripanocidal drug candidate by its exclusive action on protozoan.

Keywords *T. cruzi*, doença de Chagas, eupomatenoid-5

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Vitamin utilization pathways as antimalarial drug targets

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During its intraerythrocytic stage, the human malaria parasite *Plasmodium falciparum* needs to acquire a number of nutrients from the extracellular environment in order to support its rapid rate of proliferation. Among these nutrients are members of the water-soluble vitamin B complex, namely pantothenate (vitamin B_5) and thiamine (vitamin B_1). We have now discovered that an extracellular supply of riboflavin (vitamin B_2) is also essential for the intraerythrocytic development of the parasite. Each of these vitamins is taken up by the parasite and converted into cofactors which play critical roles in cellular metabolism. We have therefore set out to identify analogues of these vitamins that inhibit parasite growth *in vitro* and which may thereby help us to identify novel antimalarial drug targets. Data will be presented on a number of analogues, including roseoflavin (an analogue of riboflavin), oxythiamine (an analogue of thiamine) and HoPan (an analogue of pantothenate). The antiplasmodial potency of these three analogues can be shifted by varying the extracellular concentration of the corresponding vitamin, consistent with the analogues inhibiting parasite growth specifically by interfering with the parasite's ability to utilize the vitamin. A number of the vitamin analogues inhibited parasite growth via a mechanism which is non-competitive with the corresponding vitamin. Data will also be presented on the effect of the analogues on the uptake and metabolism of the vitamins. We show that at least some of the analogues inhibit parasite growth via a mechanism which involves the analogues being converted into toxic antimetabolites. Importantly, we show that roseoflavin and oxythiamine inhibit parasite growth *in vivo* in a mouse model of malaria, providing evidence that targeting the parasite's requirement for extracellular vitamins may be a viable antimalarial strategy.

Keywords malaria; *Plasmodium*; vitamins; antimetabolites; cofactors; uptake; metabolism

7. Resistance and Susceptibility

ΦSsUD.1, a new element carrying antibiotic [*tet*(W), *erm*(B), *aadE*, *sat4*, and *aphA*] and heavy metal [*cadC/cadA*] resistance genes detected in a human isolate of *Streptococcus suis*

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tet(W) is an emerging tetracycline resistance determinant, associated either with conjugative or nonconjugative elements, whose host range is second only to that of *tet*(M) among ribosomal protection *tet* genes. We first described *tet*(W) in the emerging zoonotic pathogen *Streptococcus suis*, in human and pig strains isolated in Italy. The *tet*(W)-carrying genetic element from human strain SsUD, isolated from a case of meningitis, was characterized. The element was transferable (at a low frequency) and, though apparently noninducible following mitomycin C treatment, displayed a typical phage organization and was named ΦSsUD.1. Its full sequence was determined (60,711 bp), the highest BLASTN score being Φm46.1, the main *S. pyogenes* element carrying *mef*(A) and *tet*(O) genes. ΦSsUD.1 exhibited a unique combination of antibiotic and heavy metal resistance genes. Besides *tet*(W), it contained a MAS (macrolide-aminoglycoside-streptothricin) fragment with an *erm*(B) gene having a deleted leader peptide, and a *cadC/cadA* cadmium efflux cassette. The MAS fragment closely resembled the one recently described in pneumococcal transposons Tn*6003* and Tn*1545*. ΦSsUD.1 appears to be a novel genetic element for *S. suis*, since no resistance genes have previously been detected in sequenced phage genomes. The resistance genes found in the ΦSsUD.1 phage scaffold differed from, but were in the same position as, cargo genes carried by other *S. pyogenes* phages: for instance, *cadC* and *cadA vs. mef*(A) in Φm46.1 and Φ10394.4; *tet*(W) *vs.* a restriction/modification system in Φ10394.4; and the *erm*(B)-containing MAS fragment *vs. tet*(O) in Φm46.1. The chromosome integration site of ΦSsUD.1 was at the 3' end of a conserved tRNA uracil methyltransferase (*rum*) gene. This site, known to be an insertional hot-spot for mobile elements in *S. pyogenes*, might play a similar role in *S. suis*.

Keywords prophage; Streptococcus suis; tet(W); erm(B); aadE; sat4; aphA; cadC/cadA

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A 3-Year Review on the Profile of Multidrug-resistant Gram-negative in a Tertiary Teaching Hospital in Malaysia

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Introduction: Multidrug-resistant Gram-negative organism is a threat to hospitalized patient. It is associated with significant morbidity and the cause of high mortality in patient with nosocomial infections. Infection control personnel should analyzed the profile of these organisms in the hospitals to implement appropriate measures for prevention. Here we analyzed the profile of multidrug-resistant Gram-negative in Hospital Universiti Sains Malaysia, a tertiary teaching hospital located in North-Eastern State in Malaysia.

Methodology: All significant Gram-negative isolates and their antimicrobial profiles from 2007 to 2009 were analyzed. The organisms were identified by conventional method and API E and API NE. The antimicrobial sensitivity was determined by modified Kirby Bauer method the sensitivity breakpoint were according to Clinical Laboratory Standard Institute (CLSI). Multidrug-resistant is defined as bacteria resitant to three or more classes of antibiotics.

Results: 6978 Gram negative were isolated during that period. 840 (12%) were resistant to 3rd and 4th generation cephalosporin, ciprofloxacin and piperacillin tazobactam. They were *Acinetobacter* spp. (69%), *Klebsiella pneumoniae* (14 %) and *Pseudomonas aeruginosa* (6%). Other organisms found to be resistant were *Enterobacter* spp (2%), *Escherechia coli* (2%), *Klebsiella* spp (3%) and *Stenotrophomonas maltophilia* (1%). Most of the isolates were from tracheal aspirates (35%), wond specimens (21%), blood (16%) and urine (13%). They were mostly from intensive care unit (38%) and hospitalized patient (59%). Of the hospitalized cases, most of the isolates were from Surgical and Neurosurgical Department (40%) followed by Medical and Orthopaedic Department, 27% and 13% respectively.

The sentivity pattern of these organisms sensitive to other antibiotics were to amikacin (42%), gentamicin (14.8%), netilmicin (49.5%), cefoperazone/sulbactam (74.8%), imipenem (25.7%) and meropenem (24.6%)

Conclusion: Multidrug resistant organism were high and mostly isolated from tracheal aspirates and wounds. Most of the organisms were also resistant to commonly used antimicrobials. Cefoperazone/sulbactam and amikacin exhibit the highest percentage of susceptibility.

Keywords multidrug-resistant, Gram negative infections, profile

A 5-year-period evaluation of polymyxin B against multidrug-resistant *Pseudomonas aeruginosa* clinical specimens

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Pseudomonas aeruginosa is a wide spread nosocomial pathogen that has two important features: virulence and antimicrobial resistance traits; due to these characteristics, *P. aeruginosa* causes several infections, from superficial skin infection to fulminant sepsis. Intrinsic and acquired resistance to almost all clinical antimicrobial drugs is the greatest difficult in control of *P. aeruginosa* infections. Polymyxin B (PB) and colistin are some of the last antimicrobial agents with high efficacy against multidrug-resistant *P. aeruginosa* (MDR-PA). Strains showing reduced susceptibility or even resistance to these drugs have also been reported, though. Regarding this concern, we aimed to evaluate the PB minimal inhibitory concentration (MIC) against clinical specimens of MDR-PA over a period of 5 years (2006-2010) and the PB usage on the same interval. Samples were obtained from patients attended at University Hospital of Botucatu Medical School, a public tertiary Brazilian hospital, in which this germ seems to be endemic. Specimens were isolated from patients of several wards and identified by either manual or automated system (Vitek Legacy) techniques. Antimicrobial susceptibility test was carried out by Kirby-Bauer disk-diffusion method, and the results were interpreted according to CLSI. Multidrug-resistant strains, i.e., strains that showed resistance to penicillins, cephalosporins, carbapenems, monobactam, aminoglycosides and quinolones, were submitted to PB MIC determination by the E-test (AB Biodisk). Usage of PB was provided by local pharmaceutics' register. Median values of PB MIC were calculated, year by year, and the results were compared using the Mann-Whitney non-parametric test (SPSS); average of PB mensal usage (500,000 IU flasks) was calculated yearly; tendency curves for each variable were calculated, as well as Pearson correlation index; p values below 0.05 were considered statistically significant. A total of 126 strains of *P. aeruginosa*, isolated from 88 different patients, were evaluated and all strains showed susceptibility to PB, MIC ranging from 0.094 to 3.0 µg/mL. MIC that inhibited 50% (MIC50=median) and 90% (MIC90) of the isolates were 1.0 and 1.5 µg/mL, respectively. Increasing values of PB MIC50 (R^2=0.38) and mensal average PB usage (R^2=0.8) were observed. PB MIC and mensal average PB usage did not show correlation (Pearson correlation index=0.34; p=0.58). Figure shows the MIC50 and PB usage evolutions over the 5-year period.

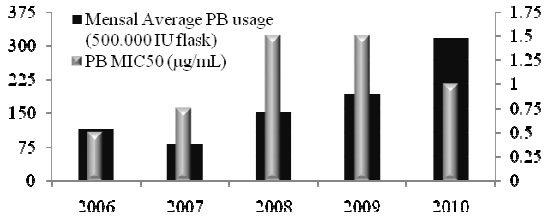

Figure. Polymyxin B (PB) MIC50 and mensal average rates over a five-year period.

IU: International Units.

Multi-drug resistant *P. aeruginosa* represents a new challenge to therapeutics. Polymyxin B and colistin are old drugs that have been used frequently against some super bugs (namely MDR-PA, *Acinetobacter* and *Klebsiella* spp.). The impact of increasing in the usage of these drugs has not been elucidated so far. We showed an increasing PB MIC50 values in a five years period, evaluating MDR-PA from one public tertiary Brazilian hospital, but there was no correlation between PB MIC50 and PB usage. Further studies analyzing the genotypic diversity of *P. aeruginosa* strains are desirable to a better understanding of this concerning situation. These informations can be helpful to provide subsidies for an efficient treatment to MDR-PA infections.

Keywords: *P. aeruginosa*; Polymyxin B; MIC; multi-drug resistance.

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A high prevalence of resistance in new tuberculosis cases of midwestern Brazil

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Tuberculosis (TB) is a serious worldwide public health problem, and drug resistance, particularly multidrug resistance (MDR) is a critical factor involved in TB control. We analyzed *M. tuberculosis* isolates from 132 new TB cases of treatment-naïve patients in Goiás, Brazil by drug susceptibility tests, partial sequencing of the *rpoB* and *katG* genes, $inhA^{C\text{-}15T}$ mutation analysis by PCR, and RFLP-IS6110 genotyping. A high frequency of drug resistance was observed in previously untreated patients (13.6% to at least one antibiotic and 6.1% MDR-TB), and a high rate DNA polymorphism was detected in these strains. These results suggest that the prevalence of resistant TB is underestimated and that resistance in new TB cases was not associated with an outbreak in this region. We recommend routine culture and susceptibility testing for all new TB cases in Goiás for the appropriate treatment and control of this disease.

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A novel regulator of bacterial persistence

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Infections by pathogenic bacteria often lead to severe chronic diseases such as tuberculosis and bacterial pneumonia. According to the Centers for Disease Control and Prevention (CDC), these diseases cause over 300 million illnesses and more than 5 million deaths each year. Treatment of chronic infections is seriously hampered by the presence within a bacterial population of a small number of cells that is capable of surviving very high doses of antibiotics. Those so called persister cells give rise to a population with the same antibiotic susceptibility as the original population and thus have not undergone resistance-conferring mutations. Persisters have become the focus of scientific research as they are held responsible for recurring outbreaks of infections when the antibiotic pressure decreases. There are multiple hypotheses on persistence but the exact cause of the phenomenon remains elusive.

Here we describe a new regulator of persistence. Conditional gene silencing of this regulator using antisense RNA causes a decrease in the persister fraction of *Escherichia coli* while overexpressing this regulator causes a 100-fold increase in the number of persister cells. The increased persister fraction is completely nullified by introducing a single point mutation. Using flow cytometry, we have shown the likelihood of transition to the persistent state to be directly correlated to the concentration of the regulator inside a single *E. coli* cell. Furthermore, overexpressing the endogenous copy of this regulator in *Pseudomonas aeruginosa* and *Vibrio harveyi* also causes an increase in persistence, implying that this mechanism of persistence regulation is functionally conserved among different bacteria.

Further characterization of this novel persister gene will aid in unravelling the persistence phenomenon and may lead to the development of new drugs to treat chronic infections

Keywords bacterial persistence; phenotypic antibiotic tolerance; single-cell analysis

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A proteomic approach to the increasing multiresistant problem in *Stenotrophomonas maltophilia*

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Stenotrophomonas maltophilia is a Gram-negative pathogen with emerging nosocomial incidence. It has been associated with several clinical syndromes, primarily in relation to the opportunistic infection of immunocompromised patients. Although not an inherently virulent pathogen, its ability to colonise respiratory-tract epithelial cells and medical-device surfaces makes it a ready coloniser of hospital settings. Antibiotic treatment of S. maltophilia is greatly hampered by extensive drug resistance. Besides more specific resistance mechanisms, its capacity to form biofilms confers the bacterium additional protection in front of the immune system, antibiotics and disinfectants. The genomes of two different strains have been recently sequenced and compared, but no expression-pattern studies have been performed yet. In this work we have compared the proteomes of M30, E77 and UV74, three newly isolated strains from decubitus ulcer, sputum and vascular ulcer, respectively, with the ATCC 13637 collection strain. We have used the DIGE technology to compare the expression pattern of these strains under conditions of exponential growth, using the pH range 3-10. Statistical analysis of DIGE gels allowed the detection of about 300 spots with significant differences between the clinical isolates and the ATCC strain and 39 differentially expressed proteins were identified by mass spectrometry. Some of the proteins identified are involved in fatty-acid metabolism and in cell-wall polysaccharide synthesis. The upregulation of these proteins in some of the clinical strains suggests a different membrane lipopolysaccharide composition, which in turn could be related with biofilm formation and pathogenesis. In addition, some outer-membrane proteins involved in the uptake of small hydrophobic compounds and extracellular proteases show also upregulation in clinical strains. Some of the differentially expressed proteins identified here have been previously shown to have a role in virulence in other bacteria, suggesting that this comparative (and quantitative) approach may be instrumental for the discovery of pathogenicity factors.

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Keywords Stenotrophomonas maltophilia; multiresistant bacteria, proteomics

AmpC genes in ceftiofur resistant *Escherichia coli* isolated from broiler faeces

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Recent reports indicate that several species of *Enterobacteriaceae* have acquired plasmids encoding *AmpC*-like beta-lactamases that hydrolyze cephalosporins. The aim of study was to study minimal inhibitory concentrations (MIC) and the presence of *AmpC* genes in ceftiofur resistant *Escherichia coli* strains isolated from poultry faeces during year 2010. Thirty eight *Escherichia coli* isolates showed MIC XG for ceftiofur 12,9 mg/L, for cefquinome 8,1 mg/L, for ceftriaxone 8,3 mg/L and ceftazidime only 3,2 mg/L. We also documented high occurence of resistance to ampicillin (100%), ampicillin with sulbactam (37%), tetracycline (86%), streptomycin (66%), enrofloxacin (31%) and cotrimoxazol (65%) and florfenicol (21%). All strains were sensitive to gentamicin, kanamycin and spectinomycin.

CMY-2 gene was detected in twelve *Escherichia coli* strains by PCR according Perez-Perez et al. (2002) and specificity of PCR products was confirmed by DNA sequencing. Two faecal *CMY- 2* positive *Escherichia coli* strains contained *CTX-M* gene also. Results showed that faeces of broilers is reseservoir of *AmpC* genes for human population.

This study was supported by slovak grants APVV-0028-07 and VEGA-0012/08.

Keywords: ceftiofur resistance, AmpC, *Escherichia coli*, broilers

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Antibacterial Efficacy of Nisin, Pediocin 34 and Enterocin FH99 against *L. monocytogenes, E. faecium* and *E. faecalis* & Bacteriocin Cross Resistance and Antibiotic Susceptibility of Their Bacteriocin Resistant Variants

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The Gram positive bacterial species studied in this work differed considerably in their sensitivity to Pediocin 34 and Enterocin FH99. *L. monocytogenes* MTCC 657, *E. faecium* DSM 20477 and *E. faecium* (VRE) were sensitive to all the three bacteriocins used in the study. Whereas *E. faecalis* ATCC 29212 was sensitive only to nisin. *L. monocytogenes* strain showed an initial decrease in the viable counts followed by the regrowth of the survivors after 24 hours in the presence of each of the bacteriocins. Similar results were observed for other bacterial species used in the study. Bacteriocin Pediocin 34 proved to be more effective than Enterocin FH99 in inhibiting *L. monocytogenes* MTCC 657. A greater antibacterial effect was observed against *E. faecium* DSM 20477, *E. faecium* (VRE) and *L. monocytogenes* MTCC 657 when the bacteriocins were combined in pairs indicating that the use of more than one Lactic Acid Bacteria (LAB) bacteriocins in combination may be effective in inhibiting/ reducing the survival of pathogens. Bacteriocin resistant variants of *L. monocytogenes* MTCC 657, *E. faecium* DSM 20477, *E. faecium* (VRE) and *E. faecalis* ATCC 29212 were developed. Bacteriocin cross-resistance and the antibiotic susceptibility of wild type and their corresponding resistant variants were assessed. Pediocin 34 resistant variant of *L. monocytogenes* MTCC 657 displayed cross resistance to Enterocin FH99 but not to nisin. On the other hand its Enterocin FH99 resistant variant was sensitive to both nisin and Pediocin 34. Nisin resistance in *E. faecium* DSM 20477 conferred cross resistance to both Pediocin34 as well as Enterocin FH99. Enterocin FH99 resistant variant of *E. faecium* DSM 20477 displayed cross resistance to Pediocin 34 and Pediocin resistant variant of *E. faecium* DSM 20477 showed cross resistance to Enterocin FH99. Similar results were observed in case of *E. faecium* (VRE). Nisin, Pediocin34 and Enterocin FH99 resistant variants of *E. faecium* (VRE) displayed resistance to polymixin B. On the other hand Pediocin 34 resistant variant of *L. monocytogenes* MTCC 657 showed cross resistance to gentamycin and erythromycin. Nisin resistant variant of *E. faecalis* ATCC 29212 displayed cross resistance to gentamycin and kanamycin. Nisin resistant variant of *E. faecium* DSM 20477 appeared to be more susceptible to the antibiotics ampicillin, rifampicin, penicillin G, chloramphenicol and novobiocin. Nisin resistant variant of *E. faecium* VRE appeared to be more susceptible to the antibiotics vancomycin and penicillin G, and resistant to polymyxin B. In an attempt to clarify the possible mechanisms underlying bacteriocin resistance in *L. monocytogenes* MTCC 657, *E. faecium* DSM 20477, *E. faecium* (VRE) and *E. faecalis* ATCC 29212, surface properties such as cell surface hydrophobicity were analyzed and compared between the wild types and the nisin resistant strains. According to the hydrophobicity measurements significant differences ($p < 0.001$) were observed between wild type *E. faecium* DSM 20477*, E. faecium (VRE), E. faecalis ATCC* 29212 *and L. monocytogenes MTCC* 657 and their Nisin resistant, Pediocin 34 resistant and Enterocin FH99 resistant counterparts, respectively. On the contrary, Nisin resistant variant of *L. monocytogenes* was more hydrophobic ($p < 0.001$) than the corresponding wild type, whereas the Pediocin 34 and Enterocin FH99 resistant variants were less hydrophobic than the wild type strain**.** Nisin, Pediocin34 and Enterocin FH99 resistant variants of *E. faecium* DSM 20477 were less hydrophobic than their wild type counterparts. Similar results were obtained for *E. faecium* VRE and its bacteriocin resistant variants. Also, nisin resistant *E. faecalis* 29212 was less hydrophobic than its wild type counterpart.

Keywords: Nisin**,** Pediocin34, Enterocin FH99, Antibacterial efficacy, Bacteriocin Resistance

Antibiotic resistance in faecal bacteria (*Escherichia coli*, *Enterococcus* spp.) in feral pigeons

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Antimicrobial substances have been used for both treatment and prevention of bacterial diseases for decades. Due to their incorrect usage, bacteria have developed resistance to these substances. Recently, the prevalence is increasing of antimicrobial resistance in serious bacterial pathogens, such as salmonellas. Common commensal intestinal bacteria such as *Escherichia coli* and enterococci (*Enterococcus* spp.) are used as indicators of faecal contamination of the environment and often also as model organisms for detecting antibiotic resistance's occurrence in both human and domestic animal populations. Our recent studies showed that antimicrobial resistance occurs also in commensal *E. coli* isolated from wild animals that are not directly affected by antibiotic treatment.

The aim of this study was to determine the presence of antibiotic-resistant faecal *Escherichia coli* and *Enterococcus* spp. in feral pigeons (*Columba livia* forma domestica) in the Czech Republic. Cloacal swabs of feral pigeons collected in the city of Brno in 2006 were cultivated for antibiotic-resistant *E. coli*. Antibiotic-resistance genes, class 1 and 2 integrons and gene cassettes were detected in resistant isolates by polymerase chain reaction (PCR). The samples were also cultivated for enterococci. Species status of enterococci isolates was determined using repetitive extragenic palindromic-PCR. Resistance genes were detected in resistant enterococci by PCR.

E. coli isolates were found in 203 of 247 pigeon samples. Antibiotic resistance was recorded in three (1.5%, $n_{E. coli}$=203) isolates. Using agar containing ciprofloxacin, 12 (5%, $n_{samples}$=247) *E. coli* strains resistant to ciprofloxacin were isolated. No ESBL producing *E. coli* isolates were detected. A total of 143 enterococci were isolated: *Ent. faecalis* (36 isolates), *Ent. faecium* (27), *Ent. durans* (19), *Ent. hirae* (17), *Ent. mundtii* (17), *Ent. gallinarum* (12), *Ent. casseliflavus* (12), *Ent. columbae* (3). Resistance to one to four antibiotics was detected in 45 (31%) isolates. Resistances were determined by *tetK*, *tetL*, *tetM, tetO, aac(6´)aph(2´´)*, *ant(4´)-Ia*, *aph(3´)-IIIa*, *ermB, pbp5, vanA* and *vanC1*genes.

Escherichia coli and *Enterococcus* spp. isolates resistant to antimicrobial agents, including antimicrobials that are important for human chemotherapy like quinolones, cephalosporins and aminoglycosides, were observed in feral pigeons in our study. The findings of various antimicrobial resistant bacteria from feral pigeons are alarming, particularly if the genes encoding these phenotypes can be transferred into other pathogenic bacteria. Based on the results of this study, feral pigeons should be considered a risk species for the spreading into the environment of antimicrobial resistant bacteria, including antibiotic resistant *E. coli* strains and vankomycin resistant enterococci with *vanA*.

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Keywords antibiotics, antibiotic resistance, Escherichia coli, Enterococcus, vanA, feral pigeons

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Antibiotic resistance in *Pseudomonas aeruginosa* from clinical specimens: Mechanisms and phenotypic detection methods

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Background: *P.aeruginosa* is one of the leading pathogen worldwide.Nosocomial infections caused by this organism are difficult to treat because of the intrinsic resistance of the species as well as its ability to acquire resistance to multiple classes of antimicrobials. *P. aeruginosa* represents a phenomenon of antibiotic resistance and practically demonstrates all known enzymic and mutational mechanisms of bacterial resistance. The increasing prevalence of nosocomial infections caused by multi-drug resistant *Pseudomonas aeruginosa* severly compromises the selection of appropriate treatments and is, therefore, associated with significant mortality and morbidity.

Methods: A total of 83 non-repetitive isolates of *Pseudomonas spp* from various clinical samples were collected and identified using standard microbiological procedures. Antibiotic susceptibility testing was done by Modified Kirby-Bauer method. *Pseudomonas aeruginosa* ATCC 27853 was used as the control. MIC for imipenem was done by Agar dilution method. Imipenem resistant isolates were further tested for metallo-betalactamase (MBL) production by Modified Hodge test and Imipenem-EDTA double disk synergy test. Isolates were also evaluated for Amp-C production by cefoxitin disk method.

Results: Sixty three (75.9%) out of 83 isolates were susceptible to Imipenem by disk diffusion method but only 72.28% (60/83) were sensitive to Imipenem by agar dilution. *Pseudomonas spp*. showing imipenem resistance was predominantly isolated from urine samples (43.47%). Three (3.6%) out of 83 isolates were found to produce Amp C enzyme. Out of which two isolates were from urine sample and one isolate from wound swab. None of the isolate were found to produce metallo betalactamase enzyme by modified Hodge test but 17[73%] isolates out of 23 were found to produce metallobetalactamase by imipenem + EDTA double disk synergy test.

Conclusion: *P. aeruginosa* seriously contributes to hospital infections, particularly in burn

wound unit and catherized patients. Imipenem, Meropenem,Amikacin were found to be most effective while Ceftazidime, Ciprofloxacin and Gentamicin showed maximum resistance in our setting. Our study proves the increasing prevalence of multi-drug resistant *Pseudomonas* and therefore, methods to detect various types of resistance should be performed routinely. This helps in early identification of these 'superbugs' and demands effective infection control measures to control their spread.

Key words: *Pseudomonas*, Metallo-betalactamase, Amp-C

Antibiotic resistant strains in urban sewage

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The antibiotic resistant strains represent a serious concern for the public health, which may become a severe problem whether the resistant strains will be spread in the environment. Since the urban sewage is released in the environment after treatment, we studied whether antibiotic resistant bacterial strains exist in urban sewage. Urban samples were collected from area of thessaloniki (Greece) and the bacterial susceptibility against a panel of 8 antibiotics (ampicillin 50 μg/ml, cefepime 30 μg/ml, tetracycline 30 μg/ml, kanamycin 30 μg/ml, erythromycin 15 μg/ml and 75 μg/ml), rifampin 5 μg/ml, trimethoprim/sulfamethoxazole 1/5 μg/ml and ciprofloxacin 5 μg/ml) was determined for two bacterial groups (A:enterobacteria and B: Staphylococci and Streptococci) relatively with the total bacterial number in the samples. The results showed the followings: (i) almost totally resistance was observed in both groups for erythromycin, and additionally for kanamycin in group B. For the rest of antibiotics significantly higher resistance was observed in group B (0.2 -7.9%) comparatively with group A (0.001 to 0.083%). The most common antibiotic resistant strains were 14 for group A and 7 for group B, and that were isolated and then tested for their susceptibility against the 8 antibiotics. The results showed that: (i) the 14 isolates of group A were all resistant to erythromycin and rifampin, resistance to ampicillin and trimethoprim/sulfamethoxazole was displayed by one isolate and to kanamycin by two isolates. Various degree of resistance was observed for cefepime, ciprofloxacin and tetracycline. (ii) One isolate from group B was resistant to all of the antibiotics used. Resistance to kanamycin and trimethoprim/sulfamethoxazole was displayed by one isolate. Various degree of resistance was observed for the rest of antibiotics tested. Additionally these isolates were also tested for their susceptibility to1000 fold higher concentrations of antibiotics and the results showed that: (i) three isolates from group A were resistant to ampicilin, two were resistant to both erythromycin and trimethoprim/sulfamethoxazole and one to erythromycin, (ii) one isolates from group B was resistant to trimethoprim/sulfamethoxazole and one to kanamycin, (iii) the rest of the bacteria of both groups exhibited various degree of susceptibility to the antibiotics used. The above results showed that in urban sewage multi-resistant bacterial strains exist, some of which display extremely high resistance to certain antibiotics.

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Antibiotic susceptibility of *Campylobacter* spp. isolates from quail (*Coturnix coturnix*) samples collected in a Portuguese slaughterhouse

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Campylobacter infection is the most commonly reported cause of acute gastroenteritis in Europe and North America. Most infections are caused by *Campylobacter jejuni* and to a lesser extent by *Campylobacter coli.* The pathogenic organism cause watery diarrhea and/or hemorrhagic colitis associated with abdominal pain and fever. Infections are generally self – limiting with symptoms resolving in 3 or 5 days, however chronic infection could persist being *Campylobacter* also associated to the Guillain – Barré syndrome. The antibiotic therapy is used if symptoms worsen or persist frequently on children and adolescent, and particularly in immunocompromised patients, in the case of bacteraemia. At Portugal, is reported an increase of 5.3% on quail production (INE, 2010). In fact quail meat is very appreciated by Portuguese consumers being frequent the detection of *Campylobacter* in this meat. For this reason it is important to evaluate the *Campylobacter* spp. isolates susceptibility to the main antibiotics groups used in veterinary and human treatment proposes. The collection of quail samples was made during 2009 in a Portuguese slaughter house, on different working days and different flocks producers. Sampling was performed for intestine (cecae), skin neck carcass and breast of quail carcass. *Campylobacter* spp. isolates from the different samples were obtained from detection method according to ISO 10272 – 2 (2005). All isolates were identified by multiplex PCR. *Campylobacter coli* (n=46) and *Campylobacter jejuni* (n=5) isolates from quail samples were characterized concerning its susceptibility to eleven antibiotics by the disc diffusion test according recommendations of the *Comite de l'antibiogramme de la Societe Francaise de Microbiologie* (2010). Most of the *Campylobacter coli* isolates were resistant to ciprofloxacin (98 %), ofloxacin (89 %) and norfloxacin (80%). Resistance to erythromycin and tetracycline were observed on 94% of the isolates. The frequency of resistance to the other antibiotics studied was variable 94% to ampicillin, 63% to augmentin, 39% to trimethoprim + sulfamethoxazole and 13% to gentamicin. Regarding the *Campylobacter jejuni* isolates (n=5) was observed a high resistance level to ofloxaxin, ciprofloxacin, tetracycline and ampicillin (100%). This isolates reveal lower lever of resistance to Norfloxaxin (60%), erythromycin (40%) and gentamicin (20%). All *C. jejuni* isolates were susceptible for trimethoprim + sulfamethoxazole. The chloramphenicol was active to both *Campylobacter* species analyzed. The resistance to nalidixic acid was 100% to both Campylobacter species. The high rates of *Campylobacter* isolates resistance to antibiotics from quail samples at slaughterhouse level make advisable a well stated policy for the use of antibiotics and the accomplishment of all preventive rules of good hygiene practices at farm level (producers) as well the good implementation of HACCP regarding the hazard *Campylobacter* at slaughter house level.

Keywords: *Campylobacter,* Quail, antibiotic resistance, safety, poultry.

Antibiotic-resistance profile in environmental bacteria isolated from wastewater treatment plant and the receiving river

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Antibiotic resistance has largely been studied in clinically relevant bacterial pathogens. It is now evident that the environment is a huge reservoir of antibiotic-resistant bacteria, and these bacteria may be able to spread their genes to pathogens.

The aim of this study was to determine the antimicrobial susceptibility of bacteria isolated from wastewater treatment plant effluent, as well as from downstream and upstream areas of the receiving river. The resistance profiles of bacteria in the upstream river are needed to evaluate the contribution of wastewater discharge to antibiotic resistance in the downstream river samples.

One of the antibiotics commonly used in veterinary medicine or to treat some human infections is a quinolone called ciprofloxacin. Therefore, ciprofloxacin-resistant strains were isolated from the three points sampled, and then, these bacteria were tested for sensitivity to 8 different antibiotics.

The results revealed that the strains belonged to the genus A*eromonas* showed the highest percentage of ciprofloxacin-resistant isolates, ranging between 26.5 and 42.0%, whereas other groups, such as coliforms and *Cytophaga-Flavobacterium-Bacteroides* (CFB) phylum showed a lower range of resistance (1.09-1.74% and 2.88-3.29%, respectively).

According to the results obtained with antibiograms, the highest percentages of antibiotic resistance were found in the wastewater effluent, especially members of the genus *Aeromonas* as these species exhibited a 100% resistance against all tested antibiotics. It was observed that the resistance ratios were higher for the quinolones tested (nalidixic acid, norfloxacin and pipemidic acid), although these isolates were also resistant to β-***lactam*** antibiotics (penicillin, ampicillin, carbenicillin, amoxicillin and cephalotin) but in lower percentages. Among β-***lactam*** antibiotics the lower percentages belonged to cephalotin, the only cephalosporin tested, and the highest ones corresponded to penicillin.

In conclusion, our results point out that the ciprofloxacin-resistant strains showed multi-resistance to antibiotics, although the resistance percentages to the other classes of antibiotics were lower than to quinolones.

Keywords: Antibiotics, multi-resistant strains, wastewater.

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Antibiotics sensitivity profile of microorganisms encountered in the riverine areas of Ondo state, Nigeria

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This study shows the antibiotics susceptibility pattern and complexity of micro-organisms encountered in ecological zones of riverine areas of Ondo state. The species of organisms encountered are *Micrococcus spp.* 9 (34.6%) which was the predominant bacteria isolated from the soil and water samples. Similarly, *Pseudomonas spp*. 5, (19.2%) was from soil and water samples. *Bacillus spp.* 5 (14.3%), *Bacillus circulans,* 2, (5.7%), *Bacillus cereus* and *Bacillus subtilis* having 1 (11.1%) isolate each, was also among the major isolates from the soil samples. *Micrococcus luteus,* 1, (11.1%) was isolated from fish and crab samples. Some other groups of bacteria generally isolated were, *Proteus spp, Klebsiella spp, Streptococcus spp, Veillonela spp, E. coli* with 1 (11.1%) isolate respectively and *Proteus vulgaris* 2 constitute 22.2%. The isolates showed wide range of antibiotic sensitivity towards some of the antibiotics used including Gentamicin (10-27mm), Cotrimoxazole (up to 27mm) and Perfloxacin (up to 30mm), while Ofloxacin and Ciprofloxacin also have values up to 30mm inhibition zone. Many isolates were susceptible in good range to antibiotics during the study as recorded in the antibiogram (167 out of 171). This include *Micrococcus spp* (22.2%), *Pseudomonas spp* (18.1%) and *Bacillus spp* (16.2%) having the highest degree of sensitivity. Others are *Pseudomonas aeruginosa* and *Proteus vulgaris* (5.8%), *E. coli* (4.7%), *Bacillus subtilis* (1.8%). About 50% of the antibiotic used were effective against most the tested isolates. The results signify the need for environmental monitoring and susceptibility test before administration of antibiotics in health management systems.

Key words: Antibiotics, Microorganisms, Ondo State, Riverine, Sensitivity

Antimicrobial Activity of a Teicoplanin / Colistin Combination versus Multi-drug Resistant Clones of *Acinetobacter baumannii* Prevalent in the United Kingdom

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Acinetobacter baumannii has emerged as a formidable nosocomial pathogen. It is associated with bacteraemia, sepsis and respiratory infection in the immunocompromised and critically ill and frequently exhibits multi-drug resistance (MDRAB). The global spread of successful MDRAB clones, often resistant to all conventional Gram-negative antibiotics except tigecycline and colistin, raises serious challenges to our ability to control and treat infections with MDRAB. As virtually no new agents with anti-MDRAB activity are likely to reach the market within the next 15 years, attempts to enhance the activity of existing agents are clearly warranted. Although most MDRAB remain susceptible to colistin, clinical experience with the drug is limited and has not always been favourable. Furthermore, *in-vitro* studies suggest that the drug is not sufficiently bactericidal and that heteroresistance in MDRAB can readily emerge. Colistin is thought to act via disruption of the Gram-negative outer membrane with the resulting lipopolysaccharide and electrostatic modifications ultimately leading to lysis of the bacterial cell. This 'cell permeabilising' effect may improve the penetration of compounds usually excluded by the outer membrane such as hydrophilic antibiotics. We have assessed the ability of colistin to improve the activity of teicoplainin (a glycopeptide usually inactive against *A. baumannii*) versus a number of MDRAB clones circulating in the UK. Marked synergy was observed when colistin (0.5 mg/L) was combined with teicoplanin against 5 UK MDRAB clones (OXA-23 clones 1 and 2, South East, 'T' and 'Burn' strains) with the MIC of teicoplanin falling from > 256 mg/L to < 32 for all strains and as low as 1 mg/L for the 'T' strain. The *in-vivo* efficacy of a colistin / glycopeptide combination now warrants evaluation in an animal model of MDRAB infection. Similarly, an outcome analysis of patients who have received this antimicrobial combination may help to determine whether this could be a useful regimen in clinical practice.

Keywords *Acinetobacter baumannii*, polymyxins, glycopeptides

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Antimicrobial activity of phytochemicals and their synergy with streptomycin against pathogenic bacteria

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The purpose of the present study was to evaluate the *in vitro* antibacterial effects of different classes of important and common dietary phytochemicals (5 simple phenolics - tyrosol, gallic acid, caffeic acid, ferulic acid, and chlorogenic acid; chalcone - phloridzin; flavan-3-ol - (-) epicatechin; *seco*-iridoid - oleuropein glucoside; 3 glucosinolate hydrolysis products - allylisothiocyanate, benzylisothiocyanate and 2-phenylethylisothiocyanate) against *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Staphylococcus aureus.* Another objective of this study was to evaluate the effects of dual combinations of streptomycin with the different phytochemicals on antibacterial activity. A disc diffusion assay was used to evaluate the antibacterial activity of the phytochemicals and 3 standard antibiotics (ciprofloxacin, gentamicin and streptomycin) against the four bacteria. The antimicrobial activity of single compounds and dual combinations (streptomycin-phytochemicals) were quantitatively assessed by measuring the inhibitory halos. The results showed that all of the isothiocyanates had significant antimicrobial activities, while the phenolics were much less efficient. No antimicrobial activity was observed with phloridzin. In general *P. aeruginosa* was the most sensitive microorganism and *L. monocytogenes* the most resistant. The application of dual combinations demonstrated synergy between streptomycin and gallic acid, ferulic acid, chlorogenic acid, allylisothiocyanate and 2-phenylethylisothiocyanate against the Gram-negative bacteria. Phytochemical products and more specifically the isothiocyanates were effective inhibitors of the *in vitro* growth of Gram-negative and Gram-positive pathogenic bacteria. Moreover, they can act synergistically with less efficient antibiotics to control bacterial growth. Isothiocyanates could be an alternative to conventional antibiotics. Also the isothiocyanates and some of the phenolic acids could be complementary products to restore the effectiveness of less effective antibiotics in the control of bacterial infections.

Keywords: Antibacterial drug screening, antibiotic-phytochemicals synergy, glucosinolate hydrolysis products, pathogenic bacteria, phenolics

Antimicrobial resistance in *E. coli* O157:H7 from animal and human sources in southwest Spain

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC), particularly those belonging to serotype O157:H7, have recently emerged as important food-borne pathogens. The pathogenic capacity of STEC resides in a number of virulence factors, including Shiga toxins (Stx1 and Stx2). Healthy domestic ruminants are natural reservoirs of this pathogen, although this serotype has also been isolated from wild animals. *E. coli* strains expressing extended-spectrum ß-lactamases or quinolone resistance are a first public health concern. Although *E. coli* O157:H7 is widely considered sensitive to multiple classes of antibiotics, strains showing multiple resistances are on the rise. Horizontal transfer of antibiotic resistance genes is one of the mechanisms contributing to the increased resistance of STEC to various antibiotics. Integrons are subchromosomic units able to capture, integrate and express antimicrobial resistance gene cassettes. They can be mobilized by conjugative plasmid and transposons, playing an important role in the dissemination of these genes. The aims of this work are to study the antimicrobial susceptibility and the presence of antimicrobial resistance genes, including integron-associated gene cassettes and classical ß-lactamase genes, in *E. coli* O157:H7 strains isolated from animal and human sources in Spain. **Materials and Methods**: 64 *E. coli* O157:H7 previously characterized strains isolated in southwest Spain from cattle, sheep, human and wild animals (red deer, wild boar and rabbit) faeces and goat milk were studied. Minimum inhibitory concentrations for 23 antimicrobial compounds, including ß-lactams, aminoglycosides, sulphonamide, tetracycline and quinolones, were determined by microdilution broth and interpreted according to EUCAST epidemiological cut off values. Detection of *int*1 and *int*2 genes (class 1 and class 2 integrons respectively) and ß-lactamase genes ($bla_{\text{OXA-1}}$, bla_{TEM}, bla_{SHV}, bla_{CMY} and $bla_{\text{CTX-M}}$) were performed by PCR in all the resistant *E. coli* O157:H7 strains. **Results**: A statistically significant number of *E. coli* O157:H7 resistant strains (76.5%; 49/64) was found (Chi-square = 18.1; $p < 0{,}001$), particularly to sulfamethoxazole/trimethoprim (59.4%; 38/64), streptomycin (31.3%; 20/64), sulfamethoxazole (28.1%; 18/64), trimethoprim (25%; 16/64), tetracycline (25%; 16/64), ceftiofur (23.4%, 15/64) and ciprofloxacin (20.3%; 13/64). A 53.1% (26/64) of the *E. coli* O157:H7 strains were multi-resistant (three or more antibiotic resistances). The STR-SUL-TMP-SXT-TET phenotype with a 25% (8/32), itself or within other resistance profiles, was the most common phenotype between the multi-resistant *E. coli* O157:H7 strains. Nine class 1 and one class 2 integrons were detected and their gene-cassettes amplified from conserved regions (CS). There was a strong association between class 1 integron and STR-SUL-TMP-SXT-TET phenotype (7 out 9 strains). However, any ß-lactamase gene was detected among the resistant strains. **Conclusions**: Presence of class 1 and 2 integrons in the *E. coli* O157:H7 resistant strains may offer an explanation for their common resistance to sulfamethoxazole, trimethoprim, streptomycin and tetracycline. Particularly relevant is the, to our knowledge, first description of the occurrence of class 2 integrons in *E. coli* O157:H7 isolated from sheep. In addition, the virtual lacking of β-lactamase genes among the analyzed strains co-relates well with their low resistance to β-lactams. These findings suggest a need to establish continuing antimicrobial resistant screening for *E. coli* O157:H7 and a more prudent use of certain antibiotics during food animal production to reduce selection pressure of emerging antimicrobial-resistant phenotypes.

Keywords: *E. coli* O157:H7, wildlife, livestock animal, humans, antimicrobial resistance.

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Antimicrobial susceptibility in clinical isolates of *Staphylococcus aureus* harbouring of *mec*A and *luk*FS-PV genes in Northern Portugal

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a highly pathogenic multiple-drug resistant (MDR) microorganism that is most prevalent in the community. It has been found that MRSA strains can also contain genes that encode the panton valentine leukocidin toxin (PVL). The PVL toxin has been shown to be responsible for many of the severe clinical symptoms of infection with MRSA, such as severe necrotizing pneumonia, necrotic lesions of the skin and soft tissues among others. The aim of this study was to determine the presence of the *S. aureus* PVL toxin genes (*luk*S-PV and *luk*F-PV) in MRSA strains isolated from two hospitals in Northern Portugal during the period of 6 months by means of multiplex-PCR (mPCR) that simultaneous detects the gene that confers resistance to Methicillin, the *mecA* gene and the PVL genes. Clonality of the strains in study was evaluated by Random Amplification of Polymorphic DNA or RAPD discriminated by three primers and the dendrograms were generated by unweighted pair group method with arithmetic mean (UPGMA) analysis of the agarose gels using computational tools.

The results obtained indicated a frequency of approximately 50% of MRSA and a frequency of 9% of isolates carrying the *luk*FS-PVL gene. Regarding the susceptibility patterns of isolates under study it was concluded that for most antimicrobial agents used MRSA strains reveal to be resistant to more antimicrobials than the isolates that lack *mec*A gene. Regarding fingerprinting methods they were very informative in the sense that it was possible to observe a quite fixed number of strains among pathogen causing hospital-acquiring infection. The explanation for these findings is complex and multifactorial. The high diversity of clones suggests diverse genetic backgrounds rather than the global spread of a single clone.

PVL toxin is present several MRSA strains with increasing drug resistance mechanisms. Further research into the clinical aspects, risk factors and epidemiology of MRSA infections should be a priority.

Keywords MRSA; PVL

Antimicrobial susceptibility of bacterial species isolated in Sicily from ovine and caprine milk in 2009

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Monitoring antimicrobial susceptibility in pathogenic as well in commensal bacteria in animal is recommended by OIE. Mastitis is one of the most costly disease for the dairy industry and antimicrobials are important parts of the disease therapy. Objectives of this study were to determine susceptibility pattern of a panel of antimicrobial in several bacterial species isolated from mastitic milk in Sicily (Italy) during 2009.

Isolates were identified using conventional methods: Gram stained cell morphology, colony morphology and haemolysis on Columbia agar supplemented with 5% defibrinated sheep blod, catalase and oxidase activity; carbohydrate fermentations were studied with the API gallery. Antibiograms were performed through the Kirby and Bauer agar diffusion method. A bacterial suspension from a pure colture of the strain with a turbidity of 0.5 on the McFarland scale, was used to inoculate Müller-Hinton agar plate or Müller-Hinton supplemented with 5% of sheep blood for *Streptococcus spp.* and *Pasteurella spp*. For each strain at least seven antibiotics were tested.

The antimicrobial susceptibility was determined for a sample of bacterial isolates. Among those fifty percent isolates were *S. aureus* strains. The other isolates belonged to *Streptococcus agalactiae*, *S. dysgalactiae*, *Escherichia coli, Pseudomonas spp.* and *Pasteurella spp, Mycoplasma agalactiae.* and Corynebacteria).

Among all isolates only few strains were sensitive to all tested antimicrobial drugs. Most of isolates were resistant to more than one antimicrobial drug and, interestingly but alarmingly, several isolates were resistant to all tested antibiotic.

The interest on antimicrobial resistance in animal pathogens is acquiring interest for the exchange of mobile genetic elements with bacteria pathogenic for humans and monitoring bacterial resistance of animal pathogenic bacteria could be useful to study the spread of antibiotic resistance.

Keywords Antimicrobial resistance, *Staphylococcus aureus*, bovine, ovine and caprine milk

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Antimicrobial Susceptibility of Escherichia coli Strains Isolated From Urine Samples

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Urinary system infection is a serious health problem that effects millions of people each year. Escherichia coli, urinary tract infections are most often isolated pathogenic bacteria. In our research, pre-diagnosis of urinary tract infection urine samples taken from patients who were isolated from E.coli isolated were susceptibility to various antibiotics. Well-known classical methods of bacterial identification, BD BBL Crystal ID system is also used semi-automatic.

To determine the antimicrobial suscetibility by Kirby-Bauer disk diffusion technique with the National Committee for Clinical Laboratory Standarts recommendations were taken into consideration. Results were susceptible and resistant were rated as moderately susceptible. Total of 97 E. coli clinical isolates in our study, ceftriaxone, ceftazidime, amoxycillin clavulanate, cefuroxime and imipenem susceptibility was determined. In all 97 isolated were susceptible to imipenem. İsolates, 81 ceftazidime, ceftriaxone 72, amoxycillin clavulanate 54 and 42 isolates were susceptible to cefuroxime. İsolates used in our study the highest resistance to cefuroxime was 51 isolates. The high resistance of the isolates used in our study, 51 were found isolates cefuroxime.

Key words: Escherichia coli, antimicrobial resistance, urinary tract infections.

Antimicrobial susceptibility profile and effect of stem bark extracts of *Curtisia dentata* on multi-drug resistant verotoxic *Escherichia coli* and *Acinetobacter* spp. isolates obtained from water and wastewater samples

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Twenty one (21) *Escherichia coli* (including serotypes 026:H11, 055, 0111:NM, 0126, 044, 0124, O96:H9, O103:H2, O145:NM and O145:H2)**,** 10 *Acinetobacter lwoffii* (A1-10) and 4 *A. haemolyticus* (B1-4) strains were isolated from 32 water (18 wastewater and 14 river water) samples collected from different sources in Cape Town, South Africa. Samples were first subjected to membrane filtration and the membrane filters inoculated into MacConkey broth (MB) and Baumann's enrichment medium (BEM) and incubated at 37ºC for 24-144 h, after which a loop full of the MB and BEM were then surface inoculated onto Eosin Methylene Blue (EMB) and Leeds Acinetobacter Medium (LAM) and further incubated for 24-48 h at 37ºC in order to isolate *E. coli* and *Acinetobacter* spp. respectively. The isolates obtained were presumptively identified biochemically using oxidase test strips, sulfide indole motility (SIM) medium and Erchlich's reagent. *E. coli* isolates were then confirmed serologically using *E. coli* polyvalent (Bioweb, SA) and *Acinetobacter* spp. biochemically using RapID™ NF plus test kit (Bioweb, SA). Screening for verotoxin production using Glisa Duopath Verotoxins® (Merck, Germany) test kit showed that 71% (15) of all the *E. coli* and 75% (3) of *A. haemolyticus* isolates produced verotoxin, while none of the *A. lwoffii* produced verotoxin. Results of antimicrobial susceptibility testing using the disc diffusion method showed that all the isolates were resistant to more than four antibiotics indicating multidrug resistance (MDR), with 61% of the 21 *Escherichia coli* isolates resistant to ampicillin (10 µg), cefuroxime, cephalexin, ceftazidime and tetracycline (30 µg in each case), 44% of the 10 *Acinetobacter lwoffii* isolates resistant to ampicillin (10 µg), ofloxacin (5 µg), cefuroxime and ceftazidime (30 µg in each case), while 50% of the 4 *A. haemolyticus* strains obtained were resistant to ceftriaxone, cefuroxime, nalidixic acid, amikacin and tetracycline (30 µg in each case). All the isolates were susceptible to aztreonam (5 µg), gentamicin (10 µg), cefotaxime (30 µg) and ciprofloxacin (5 µg). Results also showed that 50-68% of the MDR isolates were susceptible when tested (disc diffusion method) against ethanol stem bark extracts of *Curtisia dentata* with MIC values ranging between 2.5-20 mg/ml. The study has revealed that verotoxic multidrug resistant *E. coli* and *Acinetobacter* prevail in the environment and that *C. dentata* stem bark extracts has the potential to provide alternative source of antimicrobials that can be used in controlling these multi-drug resistant pathogenic bacteria.

Key words: *Acinetobacter* spp.; Baumann's enrichment medium; *Curtisia dentata; Escherichia coli;* multi-drug resistance; plant extracts; verotoxins.

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Antimicrobial Utilization in Intensive Care Units of a Private Tertiary Care Hospital

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The use of antimicrobial drugs (AMDs) is a major determinant for the development of resistant microorganisms. At the same time, it is also well documented that AMDs find potential use in the Intensive Care Units (ICUs) of a hospital. ICUs represent the most frequently identifiable source of nosocomial infections within the hospital, with rates of infection and antimicrobial resistance several folds higher than in the general hospital setting. It is, therefore, appropriate that surveillance of the AMD usage is performed regularly so as to optimise the use of AMDs and decrease the adverse effects.

OBJECTIVE - To evaluate the utilization of antimicrobials in ICUs of a private tertiary care hospital using RPM Plus indicators.

METHODOLOGY - A prospective observational study was conducted at two ICUs of the hospital- Coronary Care Unit(CCU) and Surgical ICU (SICU). Admissions having more than one bed day were included in the study while admissions having no bed day or one bed day, medico-legal cases, patients of tuberculosis and patient case records with incomplete documentation were excluded.

RESULTS & DISCUSSIONS -The number of cases studied in SICU and CCU were 305 and 410, respectively. The average age of patients in SICU was 58.29±0.67 and in CCU was 63.76±0.59 years. The proportion of males to females did not differ substantially in both the ICUs. In SICU, the average number of total medications was 11.71±0.16, of which the average number of AMDs was found to be 1.32±0.04. For CCU, the average number of medications was9.37±0.15 and out of this, the average number of AMDsprescribed was 1.79±0.05. 95% of AMDs were administered parenterally in SICU while it was 78.2% in CCU; however, this difference was statistically insignificant (p=0.546). The percentage of AMD prescribed by its generic name was 13.8% in SICU and 8.9% in CCU. AMDs prescribed from the National List of Essential Medicines (NLEM) in CCU weretwo times the numbers prescribed in SICU (42.5% vs 20.8%). There was 100% compliance with respect to prescribing from the hospital formulary for both the ICUs studied.

The average age of patients admitted to CCU was found to be significantly higher when compared to SICU ($p<0.001$). In SICU, the average number of medications prescribed to patients was found to be significantly higher when compared to CCU ($p<0.001$). Inspite of the average number of medications being lower in CCU, the average number of AMDs was found to be significantly high when compared to SICU ($p<0.001$). The average number of drugs that was prescribed from the NLEM in CCU was found to be significantly high when compared to the other ICU ($p<0.001$).

Floroquinolones were the AMDs most utilised in CCU while 1st generation cephalosporins were the first choice in SICU.

CONCLUSION –AMD consumption was found to be greater in CCU and one of the possible reasons could be that the patients admitted were relatively older. There is a scope for improvement in the prescription of AMDs by generic name and also from the National List of Essential Medicines of India. 1st generation cephalosporins (cefazolin) were the most commonly used for surgical prophylaxisin SICU.

Keywords Antimicrobial utilization; Intensive care units

Assessment and genetic characterization of drug resistance in *Mycobacterium bovis* strains from animals in Sicily

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Mycobacterium bovis, the etiological agent of bovine tuberculosis (TB), has an exceptionally wide host range and a complex epidemiological pattern of infection by affecting domestic, farmed, free-ranging, and wildlife animals, as well as humans. *M. bovis* is naturally resistant to pyrazinamide and usually susceptible to most antibiotics used to treat human tuberculosis. in Italy TB is still a major concern in southern and insular regions, despite the eradication plans have been enforced for more than 10 years. A preliminary study, for evaluating the susceptibility of *M. bovis* isolates from animals in Sicily, was carried out towards the first line antibiotics used to treat human tuberculosis. On that account, 25 *M. bovis* isolates from sheep and autochthonous pigs with granulomatous lesions compatible with TB, were tested for their antimicrobial resistance to ethambutol (EB), isoniazid (IZ), rifampin (RI), streptomycin (SM), and kanamycin (KM), by following the National Committee for Clinical Laboratory Standards technical guide. The genetic characterization of resistant strains were also performed by nucleotide sequencing of target genes.

Our results indicated an high prevalence of resistant strains to EB (EB^R) which was correlated to missense mutations of EmbB, an arabinosyltransferase involved in cell wall biosynthesis.

Although limited studies describe the drug-resistance (DR) in *M. bovis*, our preliminary data highlight the importance of the drug susceptibility evaluation of *M. bovis* isolates in order to understand the magnitude of influence of the transmission of DR or multi-DR *M. bovis* strains and to ensure the implementation of the most appropriate therapy to patients.

Keywords *Mycobacterium bovis*; antimicrobial resistance

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Bacterial clearance from blood in mice infected by *S. pneumoniae* (penicillin MIC=16 µg/ml) presenting specific IgG (non-protective levels) and treated with sub-therapeutic regimens of cefditoren (a highly bound cephalosporin)

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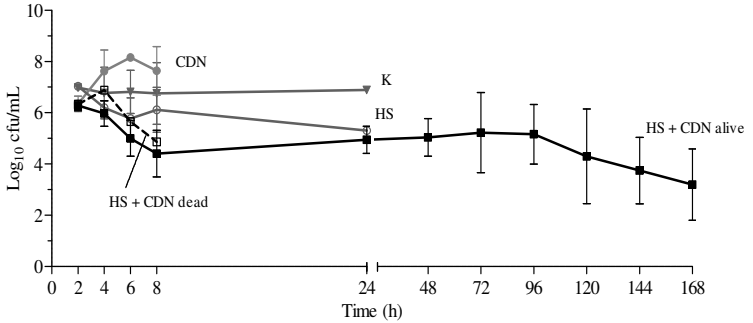
Objective: Specific antibodies, likely to be present before *S. pneumoniae* infection, can influence the activity of antimicrobials. This study explored the effect of the presence of non-protective levels of specific immunoglobulins on blood bacterial clearance by sub-therapeutic regimens of cefditoren (CDN).

Methods: A mice sepsis model was performed using a serotype 23F pneumococcus as infecting strain ($MIC_{penicillin}$=16µg/ml; MIC_{CDN}=4µg/ml). Hyperimmune serum was obtained by 5-week inoculation of a heat-inactivated inoculum. IgG levels were measured. The hyperimmune serum dilution (HS) and the tid CDN regimen over 48h producing ≤10% survival over 7 days after intraperitoneal inoculation of $4.7x10^6$cfu/ml were determined. This HS dilution and CDN regimen were administered to four groups of 10 infected animals each as follows: a) Control group (K)- received placebo (PBS), b) HS group- received HS alone 1h pre-inoculation, c) CDN group- received CDN alone initiating the 48h tid treatment 1h post-inoculation, and d) HS+CDN group- received HS 1h pre-inoculation followed by CDN 48h tid treatment starting 1h post-inoculation. Survival was recorded over 7 days. Blood samples over 168h were collected from tails and colonies (cfu/ml) were measured. CDN concentrations and protein binding were measured, and t>MIC (total and free) calculated.

Results: Mean IgG levels in the hyperimmune serum were 251 µg/ml. The non-protective dilution of hyperimmune serum was 1/4. CDN protein binding was 86.9%. The sub-therapeutic regimen of CDN was 50 mg/kg tid, with t>MIC for total and free concentrations of 22.4% and 12.7%, respectively. Survival in the combined intervention group (HS+CDN group) was 80%. The figure shows log10 cfu/ml in blood over 168h for the four study groups.



Conclusions: In the presence of non-protective specific IgG levels, the sub-therapeutic CDN regimen provided therapeutic efficacy, significantly decreasing bacteremia and mortality in mice infected by highly resistant pneumococcus

Can class IIa bacteriocin sensitivity be predicted by bacterial surface properties caracterisation?

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The class IIa bacteriocins are ribosomally synthetized heat-stable antimicrobial peptides of small molecular weight. They exhibit a cationic and largely hydrophilic N-terminal domain separated from the more hydrophobic/amphiphilic C-terminal domain by a hing. The mechanism of action of class IIa bacteriocins is generally described as composed of two steps. The first step is the adsorption of the unstructured peptide on the cytoplasmic membrane by electrostatic interactions and/or by affinity interaction with a receptor. The second step was the formation of the α-helix motif in the C-terminal leading to the bacteriocin penetration inside the membrane of the target cell.

Since the adsorption and/or the α-helix motif formation seem to rely on the surface properties, these properties were studied for different bacterial strains sensitive or resistant to two carnobateriocins, Cbn BM1 and Cbn B2, by two distinct approaches, the microbial adhesion to solvent on one hand and the bacterial electrophoretic mobility on the other.

The microbial adhesion to solvents method was used to determine the hydrophobicity and the Lewis-acid/base general properties of the bacterial surfaces. The percentage of adhesion to apolar solvent is near zero, indicating that the majority of the strains tested present an hydrophilic surface. Percentage of adhesion to chloroforme, éthylacetate and dietylether indicate a bipolar character for *Listeria* and *Enterococcus* strains, whereas *Leuconostoc* strains exhibit a Lewis acid character.

The electrophoretic mobility measurements were performed in solutions of different strength values. According to the soft particle model, the cell surface electrical properties of the strains were determined. The softness parameter (λ_0^{-1}) and the volumic charge (ρ_0) obtained from the fitting procedure indicate great variations among the various bacterial strains. *Leuconostoc* and *Enterococcus* strains present generally the lowest volumic charge.

Although these tools brought relevant informations in other fields of investigation, such as bacterial adhesion and pathogenicity, they can't lead to establish a strong link between the bacterial surface properties and the bacteriocin resistance/sensitivity property of a given strain.

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Candidemia: Treds in Candida albicans distribution and antifungal susceptibility

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Within the genus *Candida*, the species *Candida albicans* is the most common fungus isolated from humans and accounts for 50 to 70% of all nosocomial bloodstream infections. The aim of this study was to determine the antimicrobial susceptibility of clinical and environmental isolates by the technique microdilution in broth according the document CLSI (M27A2); in addition to phenotypic methods for characterizing isolates of *Candida albicans*, DNA-based methods, Random amplified polymorphic DNA (RAPD) was used to better characterize the genotypic relatedness among them. From 98 patients, 119 candidemia episodes was proven by blood culture (Becton Dickinson BACTEC 9240): *C. albicans* occurred in 35 (30%) clinical isolates; the basic methods used to name the isolates were carbohydrate assimilation (API20C kit; Analytab, Plainview,N.Y.), germ tube formation in serum, morphological analysis of cells grown on cornmeal agar and CHROMagar Candida (DIFCO). In cases in which unusual reactions were seen, isolates were characterized by further carbohydrate assimilation tests. In addition, environment isolates were obtained from bed of these patients, which only 4 (9%) were positive for *C. albicans*. The total genetic identity was found in 36% (N=14) of the isolates and its phenotypic correlation to the azoles (cetoconazole, fluconazole and itraconazole), as well as amphotericin B, was detected for nine isolates from patients located at the distinct geographic, in these cases, the profile of dose-dependent susceptibility to the itraconazole occurred for 4 isolates (3 clinicals and 1 environment). For epidemiological studies, molecular methods that are focused on the evaluation of DNA profiles are increasingly being used as useful tools for the determination of the common origin of strains of *Candida* species, through the demonstration of their genetic relatedness, thus allowing the adoption of adequate prophylactic measures. Continuous investigations are aimed due to relevance of the nosocomial infections, besides is an important problem of Public Health.

Key-Words: *Candida albicans*, clonal relation, antifungal sensitivity.

Carbenicilin resistance genes from metagenomic libraries derived from microbial mats at the Cabo Rojo Salterns

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The Cabo Rojo (CR) Salterns located at the Southwestern region of Puerto Rico posses a series of microbial mats where microorganisms are subjected to extreme conditions. Most microorganisms in the mats belong to unidentified groups making them excellent ecosystems for the construction of metagenomic libraries to search for antibiotic-resistant genes. After direct cloning of DNA from the Candelaria lagoon at the CR Salterns, our group constructed a high molecular weight metagenomic library. Genomic DNA was extracted from the Candelaria microbial mats using an indirect method, and a fosmid library of approximately 33,000 clones was generated in Escherichia coli. The clones in this library carry inserts of more than 25Kbp. The library was screened for antibiotic resistance genes using plates supplemented with Carbenicillin (100µg) and Gentamycin (10µg). From the initial screening, seven clones exhibit resistance against these two antibiotics. These clones were tested for the presence of an insert, and reconfirmed for antibiotic resistance using a traditional Kirby and Bauer susceptibility assay. Only two of the clones showed resistance to Carbenicillin, LC001 and LC002. Further Minimum Inhibitory Concentration (MIC) assays against these two clones have shown resistance to more than 60,000 µg/ml of Carbenicillin. Using a transposon-mediated vector we have sequenced the genes responsible for Carbenicillin resistance. The sequence obtained from LC001 indicated a 97% similarity to a beta-lactamase inhibitor protein from *Bacillus* sp. Sequencing efforts for clone LC002 are underway to determine if novel mechanisms for Carbenicillin resistance can be reported from the microorganisms present in the CR Salterns microbial mats.

Keywords, Microbial mats, metagenomic libraries, carbenicillin resistance

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Characterization of β-lactamase resistance phenotypes and integron carriage of *Aeromonas* spp. isolated from cultured fish

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Aeromonas spp. isolates often display multi-drug resistance phenotypes, which is problematic given their involvement as fish pathogens associated with morbidity in aquaculture systems, in addition to their role as opportunistic human pathogens. The antimicrobial susceptibilities of 31 presumptively identified *Aeromonas* spp. isolates, from catfish, goldfish and tilapia, were determined by the disc-diffusion method. Plasmid content was determined by the alkaline lysis protocol. Extended-spectrum β-lactam and metallo-β-lactam resistance phenotypes were determined using the EDTA disk inhibition test, while the double disk test was used for extended spectrum β-lactamase (ESBL) detection. Integron carriage was determined by amplification of two integrase (*intI* and *intII*) genes, *qacEΔ1–sulI* genes, and amplification of the resistance gene cassette arrays using the CS and Hep primer sets. High levels of resistance to amoxicillin, ampicillin, oxacillin, trimethoprim, and sulphamethoxazole were observed for isolates, in addition to decreased susceptibility to augmentin, cefoxitin, cefpodoxime, erythromycin and tetracycline. Majority of the isolates were susceptible to fluoroquinolones, second- and third-generation cephalosporins and macrolides. Plasmids were detected in 35.5% isolates, of which 29% appeared to carry multiple plasmids. Metallo-β-lactamase resistance was not observed, however, ESBL resistance was observed for 19.4% of isolates, with cefotaxime synergy being most frequent. Class 1 and class 2 integrons were carried by 19.4% and 80.7% of isolates, respectively. Integrons were detected in all plasmid-bearing isolates. The presence of plasmids bearing integrons and/or β-lactamase genes could result in the spread of these resistance gene determinants in environmental isolates and potential zoonotic transmission. This poses a threat to the aquaculture industry as well as public health as it could lead to treatment failure, which is associated with significant morbidity and mortality.

Keywords: *Aeromonas* spp. plasmid, β-lactamase resistance, integrons

Characterization of coagulase-negative Staphylococci methicillin-resistant isolates from blood cultures in a Brazilian University Hospital

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Coagulase-negative staphylococci (CNS) are members of the normal microflora of human skin, being the predominant microorganisms isolated from clinical materials and the leading cause of bacteremia in the hospital. The increasing in methicillin-resistant *Staphylococcus* (MRS) isolated has fundamental significance in nosocomial infections, because they leave few alternatives for treatment these infections. Resistance to methicillin is mediated by gene *mecA*, which is carried on a specific mobile genetic element identified as Staphylococcal cassette chromosome *mec* (SCC*mec*). There are eight types of SCC*mec* with types I, II and III predominant in MRS isolated in hospitals, and others in community samples. In addition, virulence factors such as production of staphylococcal enterotoxins may aggravate the pathogenesis of infection caused by these bacteria. Thus, the objective of the present study was to characterize the CNS species isolated from blood cultures from patients hospitalized in the University Hospital of the Botucatu School of Medicine (HC-FMB) for the detection of MRS and staphylococcal enterotoxins genes. A total of 86 CNS strains isolated from blood cultures from patients hospitalized of the HC-FMB were studied. The species were identified by biochemical tests and the technique of Internal Transcribed Spacer – PCR (ITS-PCR). The detection of *mecA* gene was performed by PCR and positive samples for this gene were subjected to multiplex PCR technique for SCC*mec* typing. Using the PCR method were detect the genes *sea, seb, sec* and *sed* that encode the enterotoxins A, B, C and D, respectively. Of the 86 samples of CNS were identified 71% *S. epidermidis*, 9.3% *S. warneri*, 7% *S. haemolyticus*, 7% *S. capitis*, 4.6% *S. hominis* and 1.1% *S. cohnii*. The *mecA* gene was deteted in 83.7% of the CNS, being present in 85.2% *S. epidermidis*, 87.5% *S. warneri*, 83.3% *S. capitis*, 25% *S. hominis*, 100% *of S. haemolyticus* and in the only strain of *S. cohnii* studied. The SCC*mec* was typed in 63 MRS being that 45.8% were type III, 25% type I, 11.1% type IV and 5.5% type II. The search for staphylococcal enterotoxins genes detected 77 samples positive for at least one gene, and 88.4% of CNS positive for *sea* gene, 29.1% for *seb* gene, 24.4% for *sec* gene and 5.8% for *sed* gene. Of the 72 samples of MRS, 94.4% were positive for one enterotoxin gene investigated in this study. The *mecA* gene was detected in all species isolated in the study, being the *S. epidermidis* is the most isolated and with high percentage of methicillin resistance. Typing of SCC*mec* showed the prevalence of nosocomial MRS (SCC*mec* type III) in the blood cultures. The SCC*mec* type III encode the largest number of resistance genes is an important pathogen in hospitals and can cause serious infections. The characterization of SCC*mec* type IV showed the presence of community strains isolated from hospital clinical materials. A large percentage of staphylococcal enterotoxin genes in samples of MRS is a important result, because is these genes are expressed may cause a variety of toxics effects that aggravate the pathogenesis of infections caused by these microorganisms. Data from the study highlighting the importance of detection and characterization of MRS and of virulence factors to a better understand of the profile of CNS isolated in blood cultures for adequate treatment of infections caused by these microorganisms.

Keywords *Staphylococcus* coagulase-negativa; *mecA*; SCC*mec*; enterotoxins.

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Characterization of the extended spectrum β-lactamase and metallo-β-lactamase content of *Chryseobacterium* spp. isolated from cultured fish

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Yellow-pigmented *Chryseobacterium* spp. frequently occur in soil, freshwater and marine environments and are not only food spoilage organisms but also opportunistic pathogens in immune-compromised humans and fish. Their intrinsic resistance to a wide variety of currently used antimicrobial agents often creates therapeutic problems in a clinical setting. The β-lactamase phenotypes of 36 *Chryseobacterium* spp. isolates from fish, together with fourteen *Chryseobacterium* type strains was examined using disk diffusion inhibition assays. Extended-spectrum β-lactam and metallo-β-lactam resistance phenotypes were determined using the EDTA disk inhibition test, while the double disk test was used for extended spectrum β-lactamase (ESBL) detection. The β-lactamase gene content of these isolates were determined by PCR using 6 primers targeting four metallo-β-lactamase (MBL) genes (*bla*B3, bla_{GOB3}, bla_{IND-0}, bla_{IND-2}) and two ESBL genes (bla_{CGA-1}, bla_{CGB-1}) associated with chryseobacteria. MBL and ESBL activity was observed for 21.42% and 71.42% of type strains, whilst 55.55% and 75% of study isolates displayed MBL and ESBL activity, respectively. Various combinations of ESBL and MBL genes ranging from 1 – 4 were observed for chryseobacteria from fish. The most predominant β-lactamase combination was *bla*B3, bla_{CGA-1}, and $bla_{IND-0,}$ which was observed for 36.1% of isolates, followed by the *bla*B3 and bla_{CGA-1} combination in 19.4% of isolates, bla_{CGA-1} and bla_{IND-0} in 13.9% of isolates, and bla_{CGA-1} in 11.1% of isolates. Outbreaks of disease associated with *Chryseobacterium* spp. in aquaculture systems could be problematic given the high incidence of β-lactamase resistance observed in these fish isolates. β-lactam agents should therefore not be considered as a therapeutic option if chryseobacteria are the suspected pathogens or are commonly observed associated with the fish being cultured.

Keywords *Chryseobacterium* spp.; extended spectrum β-lactamase; metallo-β-lactamase

Characterization of the mechanisms of quinolone resistance in vancomycin resistant enterococci of different origins

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Objective: The aim of the study was to characterize the polymorphism in *gyr*A and *par*C genes in vancomycin-resistant enterococci (VRE) from human, animal and food origins, exhibiting different susceptibilities to quinolones, and also to analyse the mechanism of ampicillin resistance and multi-locus sequence typing of some of these strains.

Methods: Mechanisms of quinolone resistance were analysed in 22 VRE (*van*A, 5; *van*B2, 5; *van*C1, 11; *van*C2, 1) of different origins (food, 16; human patients, 5; poultry faeces, 1), and species (*E. faecium,* 9; *E. durans,* 1; *E. gallinarum,* 11; and *E. casseliflavus,* 1), which showed different susceptibilities for ciprofloxacin (MIC: 0.5->256 mg/L).). Ampicillin resistance and multilocus sequence typing (MLST) was also carried out in some *E. faecium* strains.

Results: All *vanA* or *vanB2*-containing strains with ciprofloxacin MIC of >32 mg/L presented amino acid changes in GyrA protein (S83I, S83Y, S83R or S83I-E87G) with/without changes in ParC protein (S80I or S80R or S80L). One *van*A-containing *E. durans* strain with ciprofloxacin MIC of 64 mg/L presented the S83I and S80I changes in GyrA and ParC proteins, respectively. Two *van*B2 *E. faecium* strains were typed by MLST and both were ascribed to the CC17 (ST78 and ST17-like). All seven vancomycin-resistant and ciprofloxacin-resistant *E. faecium* strains showed ampicillin resistance and the following amino acid changes in the PBP5 protein were identified: Q461K, V462K, H470Q, M485A, N496K, A499T, E525D, N546T, A558T, G582S, K632Q, P642L, E629V and P667S, together with a serine insertion at position 466´.

Conclusion: Ciprofloxacin and ampicillin resistances are detected in vancomycin-resistant *E. faecium* of the CC17 clonal complex in isolates of human and food origins.

Keywords: Vancomycin-resistant enterococci, quinolone-resistance

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Clinical and Epidemiologic Characteristics as Predictors of Treatment Failures in Uncomplicated Skin Abscesses within Seven Days after Incision and Drainage

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Abstract

Introduction Community acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has become an epidemic and is now the leading cause of superficial abscesses seen in the emergency department. Our primary aim is to determine if an association exists between three predictor variables (abscess size, cellulitis size, and MRSA) with treatment failure within 7 days after incision and drainage. Our secondary aim is to determine if an association exists between two clinical features (abscess size and size of surrounding cellulitis) and eventual MRSA diagnosis by culture.

Methods This study is a post-hoc analysis of a previously reported study evaluating the use of antibiotics in the treatment of CA-MRSA skin abscesses. Two hundred and twelve patients were enrolled in a double-blind, placebo controlled study at four military academic emergency departments. All patients received standard incision and drainage and were randomized to receive either trimethoprim/sulfamethoxazole or matched placebo. Patients returned for follow-up within 48 hours and again at one week for evaluation by physicians blinded to the treatment arm. We used bivariate and multivariate logistic regression models to examine abscess diameter, cellulitis diameter, and MRSA as predictors of treatment failure within 7 days after incision and drainage. We also evaluated cellulitis diameter and abscess diameter as predictors of MRSA.

Results Data on 190 patients were available for treatment failures within 7 days. There is no significant difference in failure rates between the patients with abscesses ≥ 5cm (26%) versus patients with < 5 cm (22%) (95% CI 8-44; p=0.53). There was no significant difference in treatment failure rates between patients who had ≥5 cm of surrounding cellulitis (27%) versus patients with < 5 cm of cellulitis (17%) (95% CI 18-35; p=0.09). MRSA positive patients however, had a significantly higher treatment failure rate (31%), compared to patients who did not grow MSRA (10%) (95% CI 22-41; p=0.002). This relationship remained significant (p=.001) when controlled for treatment with trimethoprim/sulfamethoxazole.

There was no significant difference between abscesses ≥ 5cm (55%) and < 5 cm (52%) and the likelihood of MRSA positive cultures (95%CI 33-77; p=0.51). Similarly, there was no statistically significant association between amount of surrounding cellulitis ≥ 5 cm (60%) or < 5cm (47%) and the likelihood of MRSA positive culture (95% CI 50-70; p=0.07); however, this difference may be clinically significant.

Conclusions Cellulitis and abscess size do not appear to predict treatment failures within 7 days or predict which patients will have MRSA. However, MRSA positive patients were more likely to fail treatment within 7 days of incision and drainage.

Coagulase-negative *Staphylococcus* Isolated from Neonates: Prevalence of Species, *SCCmec* Caracterization and Genotypes

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Coagulase-negative *Staphylococcus* (CNS) are responsible for 30% of nosocomial bacteremia. In neonates, they are major causes of sepsis due to invasive procedures use and low immune response to infections. The aim of this study was to evaluate the prevalence of species, antimicrobial susceptibility and clonal diversity in 84 SCN isolates from neonates of two Neonatal Intensive Care Units (NICU) in Brazil. *S. epidermidis* (61%) and *S. haemolyticus* (21%) were the most prevalent species. Oxacillin resistance was observed for 82% of isolates, including 84% of *S. epidermidis* and 100% of *S. haemolyticus*. SCC*mec* types of community origin, as types IV and V, were found in 43.5% of the isolates. The SCC*mec* IV was detected only in *S. epidermidis* species and SCC*mec* V was mainly detected in *S. haemolyticus* species. All isolates carrying SCC*mec* type III were multiresistant and belonged to the *S. epidermidis* species Analysis of the clonality performed among oxacillin-resistant isolates showed a few genotypes in one NICU, suggesting horizontal transmission of isolates, and while in other NICU large clonal diversity was observed. Most isolates of the same genotype in both the NICU showed the same SCC*mec* and similar values of oxacillin MIC, confirming the characterization of strains and their cross-transmission into NICU. Additionally, 65% of the neonates had low birthweight (<2500g), an important risk factor for acquisition of infection.

Keyword: neonates; Coagulase-negative *Staphylococcus*; bacterial identification, oxacillin resistance, SCC*mec*, clonal diversity

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Combined expression of ESBL and MBL Trait in same isolates: Environmental and Clinical Perspective

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Resistance to both third generation cephalosporins (extended spectrum β -lactamase [ESBL] producers) and carbapenems (metallo- β -lactamase [MBL] producers) is rare in the same isolates. This study was carried out to detect simultaneous expression of MBL and ESBL in the environmental and clinical isolate. Antibiogram profiles of the isolates were determined to commonly used antibiotics and confirmation of ESBLs production was carried out by the disk diffusion assay using ceftazidime in the presence and absence of clavulanic acid. Metallo β-lactamase producers were confirmed using Imipenem and EDTA disks. A total of 16 ampicillin resistant isolates obtained from tap water and soil were further screened for ESBL and MBL. Of these sixteen, five were found to be ESBL producing, two were both ESBL as well as MBL producers and remaining multidrug resistant. Similarly, we screened nearly 390 clinical isolates from a tertiary care hospital for the combined expression of ESBL and MBL and only one isolate was found to have this phenotype. All the sixteen environmental isolates and a lone clinical isolate carry plasmid and resist lead, cobalt, chromium and cadmium to varying degrees. Although, we selected only for the ampicillin resistance in environmental isolates and two of the 16 resistant organisms were found to be MBL and ESBL producers we conclude that this type of combined resistance is likely to establish first in the environmental isolates. It remains to be seen with the current practices of antibiotic subscription in India how and when such combined resistance to third generation cephalosporins and penems is going to increase and stabilize in the environmental as well as clinical isolates. Further studies are on to molecularly characterize and compare the trait in clinical and environmental isolates.

Keywords Combined expression of ESBL & MBL; Multidrug resistance;

Comparative analysis of antibiotic resistance profiles and mobile genetic markers from clinical *Escherichia coli* strains and those from diverse environmental sources in Kenya

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Clinical *E. coli* strains especially those that cause diarrhoea and urethral tract infections may find their way into the environment. Such ecosystems may serve as reservoirs for pathogens that infect through the faecal- oral route. Interplay between clinical and environmental settings may also allow bacteria from diverse environments to exchange mobile genetic elements encoding antibiotic resistance genes. We isolated 182 *Escherichia coli* strains from environmental samples (100 and 40 isolates from human and animal sewerage respectively, and 42 from soil sediment from urban slums). We also isolated 221 isolates from clinical specimen (100 from stool and 121 from urine). Antibiotic resistance profiles to major classes of antibiotics among these isolates were determined. These isolates were analysed for integrons, Tn21 and Tn7 and the plasmid profiles that are implicated in dissemination of antibiotic resistance markers. Using PCR and sequencing strategies, we analysed the cassette diversity in the integrons from the environmental isolates and compared them with those encountered in clinical isolates. Rep-PCR was used to analyse genetic relatedness of environmental and clinical isolates.

Clinical isolates were resistant to a wider array of antibiotics including cephalosporins, aminoglycosides and (fluoro)fluoroquinolones. All environmental isolates were susceptible to fluoroquinolones but 10% of these isolates were resistant to nalidixic acid. Four (4%) of the clinical strains were resistant to ciprofloxacin while resistance to tobramycin, gentamicin, kanamycin and apramycin was at 18%, 5%, 7% and 1% respectively. While resistance to various cephalosporins ranged between 3%-28% in clinical strains, none of the environmental isolates was resistant to ceftazidime, cefotaxime, cefoxitin or cefepime. Higher resistance was recorded for ampicillin (28%), tetracycline (16%), and trimethoprim (11%) sulfamethoxazole (18%) and to chloramphenicol (17%) among clinical strains but resistance to these antibiotics was at 16%, 5%, 9%, 12%, and 14% for environmental strains respectively.

The Integron class 1 was detected in 19% o and 59% of environmental and clinical isolates respectively and sequencing revealed 5 unique patterns of integron class 1 cassette arrays in environmental isolates compared to 8 arrays in clinical strains. Majority (35%) of the environmental isolates positive for integron class 1 were positive for the *aadA1* gene encoding resistance to aminoglycosides while 21% were positive for this cassette in combination with the *dfrA1* encoding resistance to trimethoprim. The aadA1+ *dfrA1* and the *aadA2+ orfF+ dfrA12* as well as *dhfrA16 + aadA2* cassette arrays were detected in both clinical and environmental strains. Other arrays detected in the clinical strains including *dfrA12 + orfF + aadA2*, *dfrA17-aadA5*, *aacA4-catB3-dfrA1* were absent in the environmental strain. There was a strong association between resistance to trimethoprim /sulphonamides and the presence of class 1 integrons ($\chi 2$, p=0.001). Two (2) urine specimens were positive for integron class 2 while none of the environmental strains harboured this element. No association was revealed between the presence of integrons and resistance to cephalosporins ($\chi 2$, p=0.70). Tn21 was closely associated with the presence of integron class 1 ($\chi 2$, p=0.002) while the 2 isolates positive for integron class 2 were also positive for Tn7. Conjugation experiments revealed that various antibiotic resistance markers including integrons and transposon Tn21 were transferred to the Sodium azide resistant *E. coli* J53. No clonal relatedness was detected among clinical or environmental strains even among those harbouring integrons with identical cassettes. The study did not reveal a predominant clonal among the strains analysed.

This study suggests that acquisition of antibiotic resistance among clinical and environmental strains is likely to be through horizontal gene transfer and not via clonal spread. There is a need to monitor the role of non clinical ecosystems as reservoirs for antibiotic resistance. The clinical isolates are likely to contain more resistance markers due to a strong antibiotic pressure in these settings.

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Comparative proteomic analysis as tool to identify virulence factors: *Helicobacter pylori*

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Helicobacter pylori is a slow growing gram-negative microaerophilic bacteria. This is one of the most important human pathogens causing a variety of diseases, such as chronic gastritis, gastric or duodenal ulcers or gastric cancer. Despite of the important number Helicobacter studies and the extraordinary large set of proteins that have been identified and related with pathogenesis the infection process is far from being well understood. In order to increase the understanding of this process we have compared the proteome of different newly clinical isolates strains against the collection strain ATCC number 700392. It is well known that *in vitro* serial passage of microorganisms causes changes in physiology and virulence factor production. It is expected then that the comparisson between clinical isolates and the collection strain shall allow us to identify proteins related with virulence. In order to perform these comparissons we have use the combination of two-dimensional electrophoresis (2DE) and mass spectrometry. 2-DE is the tool of choice to study a proteome because it delivers a map of intact proteins reflecting changes in protein expression level, isoforms or post-translational modifications. The comparisson of the 2DE maps has allowed has to identify some proteins present in the clinical isolates and not detected in the collection strain. Among the indentified proteins there are some membrane transporters as well as some proteins involved in the stress response.

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Keywords Helicobacter pylori; multiresistant bacteria, proteomics

Comparison of Different Methods for Vancomycin MICs Evaluation in *Staphylococcus aureus* Isolated from Bacteremias

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Staphylococcus aureus is the most prevalent pathogen causing bacteremia. Reduced vancomycin susceptibility in *S. aureus* isolated from patients treated with this drug has been associated with persistent bacteremia. The aim of this study was to determine the Minimum Inhibitory Concentration (MIC) to vancomycin in 140 *S. aureus* strains isolated from blood cultures of two hospitals in the city of Rio de Janeiro (Clementino Fraga Filho University Hospital, CFFUH and Marcílio Dias Navy Hospital, MDHN), between Jan/2008 and Jul/2009. Vancomycin MIC values were determined by three methods: agar dilution (AD), Etest® and the gold standard, broth microdilution (BMD). SCC*mec* types were detected in all MRSA (36%) isolates by using multiplex-PCR. BMD method found 86 (61%) isolates with MIC = 0.5µg/ml and 54 (39%) with MIC = 1µg/ml. While, AD method found eight (6%) isolates with MIC = 0.5µg/ml, 116 (83%) MIC = 1µg/ml, and 16 (11%) MIC = 2 µg/ml. Etest® method inhibited two isolates at 0.5µg/ml of vancomycin, 58 (41%) at 1µg/ml, 77 (55%) at 2µg/ml and three isolates in 3µg/ml. The concordance between the AD and Etest® methods with the BMD test was 38% and 10%, respectively. Only 11 (8%) isolates were concordant in the three tests used. Among MRSA isolates, 72% from CFFUH presented SCC*mec* type IV and 70% from MDHN the SCC*mec* type II. Only one isolated with SCC*mec* type III was observed. Using the BMD, considered the gold standard method, no isolate presented MIC values higher than 1µg/ml. No relationship between the vancomycin MIC and the type of SCC*mec* was observed. The AD and Etest® methods erroneously detected isolates with MICs of 2 and 3µg/ml, values that can make difficult the treatment with vancomycin in staphylococcal infections.

Keywords: *S. aureus*, bacteremia, vancomycin, MICs, broth microdilution, SCC*mec*

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Comparison of resistance profile and phage type of *Salmonella* Typhimurium isolated from pigs, poultry, pork, broiler meat and humans in Belgium between 2001-2006

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Antimicrobials are used both in human and veterinary medicine to treat bacterial diseases. However, their use causes selection of resistant bacteria. Antibacterial resistance is a worldwide emerging issue for both human and animal health. In Belgium several public institutions (FASFC, WIV-ISP and CODA-CERVA) collect data on serotype, phage type and phenotypic resistance (disk diffusion method) of *Salmonella* spp. isolated from animals (no phage type determination), from food and from humans.

This study concerns the comparison of antibiotic resistance data (ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline, trimethoprim & sulfonamides, nalidixic acid and cephalosporines) of *Salmonella* Typhimurium isolated from pig faeces (n=581), poultry faeces (n=196), pork (n=255), poultry meat (n=43) and from humans (n=1870) between 2001 and 2006. Also the resistance profiles and the combination of the resistance profile and the phage type were compared. The purpose was to evaluate whether these data confirm the hypothesis of a transfer of resistance from food producing animals (pigs / poultry) via the consumption of meat (pork / poultry meat) to humans. The genotypic comparison was not included in the scope of this study.

Resistance for the isolates from pigs, poultry, pork, broiler meat and humans was commonly found against ampicillin, chloramfenicol, streptomycin, sulfonamides and tetractycline (between 20,0% and 76,7%). Resistance against the combination trimethoprim & sulfonamides and against nalidixic acid was lower (between 13,3% and 20,9% and between 0,8% and 6,6%, respectively). Resistance against cephalosporines and fluoroquinolones was reported sporadically (< 1%).

The high number of human *Salmonella* Typhimurium (92,1 %, 79,0%) showing the same resistance profiles as the meat (pork and poultry meat, respectively) and animal isolates (pigs and poultry, respectively) (Table 1) supports the hypothesis of a resistance transfer from animals via consumption of meat to humans. Comparison of the combination of resistance profile / phage type between human isolates and meat isolates shows that more than 80% of the meat isolates has a combination also found in the human isolates.

These data confirm that both pork and poultry meat are an important source for transfer of resistant *Salmonella* Typhimurium to humans. A relative contribution to the transfer of resistance from both meat products to humans cannot be made based on these data, since no comparable meat contamination data are available.

Table 1. Percentage of *Salmonella* Typhimurium with a resistance profile found in isolates from animals, meat and humans and percentage of *Salmonella* Typhimurium with a combination resistance profile-phage type found in meat isolates and humans. (ND: not determined)

	Pigs (%)	**Pork (%)**	**Human (%)**
Resistance profile	74,5	94,1	92,1
Resistance profile-phage type	ND	83,3	61,3
	Poultry (%)	**Poultry meat (%)**	**Human (%)**
Resistance profile	53,6	90,7	79,0
Resistance profile-phage type	ND	87,8	27,1

Keywords Transfer of resistance, animal, meat, human, pork, poultry, *Salmonella* Typhimurium

Detection of clinic isolates of *Staphylococcus aureus* oxacillin-susceptible mecA positive (OS-MARSA) in a Health Department of Valencia, Spain.

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MARSA) has been defined as *S. aureus* showing a minimum inhibitory concentration (MIC) of oxacillin higher than 4 mg/l or having the *mecA* gene, regardless of their antibiotype. In order to determinate the true prevalence of MARSA isolated and to improve control measures to prevent the spread, it's necessary to determinate correctly the existence of de *mecA* gene, because resistance phenotypes are not always well interpreted. Currently the detection of *mecA* gene by PCR (polymerase chain reaction) is considered the reference method, although not routinely performed (Batista Díaz *et al*; 2008).

Objective: Analyze clinic isolated of *S. aureus mecA* gene carrier.

Materials and Methods: During a one year period (March 2007-March 2008) there were studied 129 samples of *S. aureus* from 120 patients, selected among 256 due to it clear clinical significance, of nosocomial and community. The more frequently samples where abscess (32%), followed by respiratory origin samples (23%) and blood samples (21%). Identification and sensitivity study performed by semi-automated methods Walkay-Away (Siemens) and Vitek2 (Biomérieux), which use the oxacillin and cefoxitin to determine the strains MARSA. The presence of the *mecA* gene by PCR was analyzed in all strains, following the protocol described by Geha *et al*. (1994). The samples were lysed with lysostaphin (Sigma) and DNA was extracted using the *Genomic Bacterial Genelute System* (Sigma).

Results and discussion: According to data from the sensitivity study 76 isolates (60%) were MSSA (methicillin sensitive *S. aureus*) and 53 (40%) were MARSA. All samples with methicillin-resistant phenotype had the gene *mecA*. In two of the strains classified as MSSA, with MIC for oxacillin of between 0.25 to 2 mg/ml and susceptible to cefoxitin, was detected the presence of *mecA* gene, these isolates are known as OS-MARSA. The two strains had been isolated from abscesses of community.

Conclusions: Treatment of these isolates OS-MARSA with ß-lactam antibiotics could induce the appearance of strains with a high level of resistance (Hososaka *et al*.; 2007). It is important to remember the existence of these strains when therapeutic failures occur in MSSA isolates treated with ß-lactam.

Keywords: OS-MARSA. *mecA* gene.

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Diffusion of extended-spectrum β-lactamase producing *Enterobacter cloacae* in a kidney transplantation unit

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Enterobacter cloacae is an increasingly important nosocomial pathogen. It has been associated with several outbreaks, usually involving strains that overproduce the chromosomal AmpC cephalosporinase or uncommonly, strains expressing extended spectrum β-lactamases (ESBLs). Until 2008, only sporadic cases of ESBL producing *E. cloacae* have been identified in our hospital. In 2009, 26 non-repetitive ESBL producing *E. cloacae* were isolated in a kidney transplantation unit.

All isolates were analysed by pulsed-field gel electrophoresis (PFGE) to establish the epidemiological relationship among them. Production of ESBL was detected by decreased susceptibility to extended spectrum cephalosporins and a positive double disk test result. ESBLs were identified by PCR amplification of $bla_{\text{CTX-M}}$, bla_{SHV} and bla_{TEM} genes and sequences analysis.

All strains were susceptible to carbapenems and cephamycins. They were resistant to fluoroquinolones, gentamicin, tobramycin and trimethoprim + sulfamethoxazole but variably resistant to netilmicin, amikacin and tetracyclines. PFGE revealed 24 unrelated profiles. All isolates carried a single ESBL (CTX-M-15: n=22 and SHV-12: n=4).

Our results showed a multiclonal outbreak. Diffusion of *E. cloacae* producing CTX-M-15 ESBL in a kidney transplantation unit is the consequence of dissemination of identical or related plasmids harbouring CTX-M-15 gene among different clones. Early detection and prompt containment can limit the spread of these multiresistant pathogens.

Keywords: *Enterobacter cloacae,* nosocomial pathogen, multiclonal outbreak

Discovery of a novel bovine-associated methicillin-resistant *Staphylococcus aureus* strain within the UK dairy herd

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The emergence in recent decades of methicillin-resistant *Staphylococcus aureus* (MRSA), and its subsequent spread in hospitals, has provided one of the major challenges for infectious disease medicine. The development of methicillin resistance in *S. aureus* is attributed to the acquisition of a mobile genetic element (SCC*mec*) (1), carrying a gene (*mecA*) encoding a penicillin-binding protein (PBP2a) with low affinity to beta-lactam antibiotics (2). Up until recently the problem of MRSA has been limited to humans, however the recent discovery of MRSA in pigs, and subsequent transmission to humans (3), has highlighted the risk that animals in the food chain pose to public health.

Beta-lactam antibiotics are used extensively in dairy herds for the treatment and prevention of bovine mastitis (4). This is likely to provide an environment that is strongly selective for the emergence of new MRSA strains. During a study investigating the transmission of bovine *S. aureus* in the UK, we identified a novel MRSA strain from milk samples. On initial screening this strain demonstrated the phenotypic characteristics of a heterogeneous MRSA, but with no evidence of the presence of a *mecA* gene or production of PBP2a. To investigate the genetic basis for the beta-lactam resistant phenotype, the whole genome of this cattle-associated MRSA was determined. This revealed the presence of a unique SCC*mec* element carrying a novel *mecA* gene together with regulatory genes (*mecI* and *mecR1*), a gene for a beta-lactamase, an arsenical resistance operon, and genes for site specific recombination (*ccrA* and *ccrB*). The development of a new diagnostic test for MRSA has demonstrated that this variant *mecA* is geographically widespread in the UK dairy cow population, and was also found in a human isolate. Strain typing and phylogenetic studies of the human isolate and a set of 24 methicillin-resistant bovine *S. aureus* collected from clinical mastitis samples, indicated that the novel SCC*mec* containing the divergent *mecA* gene can be found in five separate lineages, of which three are related.

The emergence of MRSA in this animal reservoir is a cause of considerable concern, not least because routine molecular typing methods failed to detect the *mecA* gene within this phenotypically resistant *S. aureus*, but also because this is the first report of *mecA*-positive methicillin-resistant strains in the UK dairy herd. Further work to produce mutations in this new *mecA* gene is underway to show that the possession of the divergent *mecA* gene is unequivocally associated with the phenotypic methicillin resistance. The existence of this new *mecA* gene raises important questions about the potential transfer of resistance genes between pools of human and animal pathogens, and the development of antibiotic resistance in farm animals.

Keywords MRSA, mastitis, farm animals, SCC*mec*, whole genome

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Dissemination of large IncHI1 plasmids carrying $bla_{CTX\text{-}M\text{-}1}$ in *Escherichia coli* isolates from horses, flies, staff and environment in equine clinic

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Medication of both humans and animals with beta-lactam antibiotics, particularly 3rd and 4th generation cephalosporins, underlies selection of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae which belong to common causative agents of nosocomial infections. Among them, the proportion of CTX-M beta-lactamase producers has increased within the last years. Genes encoding for ESBLs are often parts of integrons, transposons and/or plasmids and, in case of multiresistant strains of enterobacteria, these transposable elements often harbour structurally unrelated resistance genes, which enables their co-selection e.g. by sulphonamides, tetracyclines or aminoglycosides. Another dangerous resistance mechanism that is recently on the increase in enterobacteria is the resistance to fluoroquinolones determined by plasmid-mediated *qnr* genes. The aim of this study was to find out the occurrence of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in equine clinic and to analyze the impact of antimicrobial treatment on the selection of resistance.

In equine clinic, faeces from horses (n=37), environmental smears (n=50), flies (n=179), personnel (n=12) and data of antimicrobial agents used for horse treatment were taken. The samples were cultivated on McConkey agar with cefotaxime (2 mg/l) to isolate ESBL-producing *E. coli.* The presence of *bla*, *qnr* genes and additional antibiotic resistance genes and integrons was tested by PCR and their transferability was determinated by conjugation. Macrorestriction profiles of all ESBL-positive isolates was designed by PFGE and phylogenetic groups were tested by PCR. Replicon typing and restriction analysis of plasmids harbouring ESBL and *qnr* genes were performed.

In the equine clinic, 12 (32%) ESBL-producing *E. coli* isolates were acquired. Seventeen (34%) isolates from the stables environment, 33 (18%) flies and one (8%) rectal swab from clinic staff were positive for ESBL-producing *E. coli*. All the ESBL isolates were multiresistant with resistance to 5 to 12 antimicrobial agents tested and their ESBL phenotype was caused by the presence of the gene $bla_{CTX\text{-}M\text{-}1}$ and belonged to phylogenetic groups A or B1. Most isolates also contained class 1 or class 2 integrons with variable gene cassettes. All *E. coli* isolates harboured large IncHI1 conjugative 235 or 280 kb plasmid containing $bla_{CTX\text{-}M\text{-}1}$, *cat*, *strA*, *sul2*, *tetB* genes and some of them were positive for *qnrS* and/or *qnrB* located on 40 or 45 kb conjugative plasmids of IncN or IncX1 groups of identical *Hinc*II restriction profiles. Molecular characterization showed that horizontal gene transfer seems to be involved in dissemination of *E. coli* with ESBL and *qnr* genes in equine clinic more likely than clonal dissemination of particular isolates.

The study illustrates that ESBL-producing *E. coli*, as well as plasmids carrying ESBL genes of clinical interrest, can be easily transferred among horses, humans and flies living in close contact. It appears that co-selection promoted by the usage of these non-betalactam antibiotics seems to be involved in selection of ESBL-producing *E. coli* in the clinic. The presence of ESBL-producing and fluoroquinolone-resistant bacteria in the hospital environment increases the health risk and raises essential questions of the control of nosocomial infections. Large numbers of these bacteria in faeces, in stables environment and flies occuring in the clinic and also demonstration of these bacteria in the samples from the clinic staff is disconcerting. The results of the study presented indicate the essential importance of infection control strategies.

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Keywords antibiotics, antibiotic resistance, Escherichia coli, ESBL, CTX-M-1, qnr genes, horses, plasmids

Diversity of genetic lineages among CTX-M-15 and CTX-M-14 producing *Escherichia coli* strains of clinical origin in a Tunisian hospital

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Fourteen broad-spectrum-cephalosporin-resistant *Escherichia coli* isolates were recovered between June and December 2007 in a Tunisian hospital. Genes encoding extended-spectrum-beta-lactamases (ESBL) and other resistance genes were characterized by PCR and sequencing. The following ESBL genes were identified: $bla_{\text{CTX-M-15}}$ (12 isolates), $bla_{\text{CTX-M-14a}}$ (one isolate) and $bla_{\text{CTX-M-14b}}$ (one isolate). The $bla_{\text{OXA-1}}$ gene was detected in 13 $bla_{\text{CTX-M}}$-producing strains and a bla_{TEM} gene in 6 of them. The IS*Ecp1* sequence was found upstream of $bla_{\text{CTX-M}}$ genes in 8 of 14 strains, and *orf477* or IS*903* downstream of this gene in 13 strains. Nine of the strains carried class 1 integrons and five different gene cassette arrangements were detected, *dfrA17-aadA5* being the most common. One of the strains ($bla_{\text{CTX-M-14a}}$-positive) harbored three class 1 integrons, and one of them contained as gene cassette a new variant of *aac*(6´)-Ib and *cmlA* genes. CTX-M-15-producing strains were ascribed to phylogroup B2 (6 isolates) and D (6 isolates). Multilocus-sequence-typing revealed 9 different sequence-types (STs) among ESBL-positive *E. coli* strains with prevalence of ST405 (4 strains of phylogroup D) and ST131 types (2 strains of phylogroup B2 and serogroup O25b). A high clonal diversity was also observed among studied strains by pulsed-field-gel–electrophoresis (11 unrelated profiles). These results indicate that emergence of CTX-M-15 in the studied hospital is due to both dissemination of resistant clones and horizontal transfer of genetic elements containing the ESBL encoding gene among diverse *E. coli* clones.

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EdpA, a probable deacetylase, affects non-inherited antibiotic tolerance and cell surface properties in *Pseudomonas aeruginosa*

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Persister cells constitute an antibiotic-tolerant subpopulation of planktonic cultures and biofilm communities of several pathogenic bacteria, such as *Pseudomonas aeruginosa* and *Escherichia coli*. These cells survive prolonged exposure to high doses of antibiotics and seriously hamper effective treatment of infections, contributing to the appearance of multidrug resistant strains. The mechanism of persistence is currently unknown, and only few genetic determinants have been shown to be involved in persistence. Based on a previously published high-throughput screening, we here present *edpA* (extracellular determinant of persistence; gene locus PA14_66140/PA5002) as a new gene involved in non-inherited tolerance in the opportunistic human pathogen *P. aeruginosa*. Primary sequence analysis identified EdpA as a member of the PIG-L or LmbE-like protein superfamily (Pfam PF02585) with predicted deacetylase activity. Following treatment with the fluoroquinolone antibiotic ofloxacin, persistence of an *edpA* mutant is strongly reduced both in planktonic cultures and in a colony biofilm model. Furthermore, the *edpA* mutant displays reduced biofilm formation on polystyrene and glass surfaces and altered colony morphology. Persistence of the *edpA* mutant could be restored to wild-type levels by extracellular complementation in a co-culture experiment. The phenotypes are most likely caused by an altered cell surface of the mutant. This is the first report of a possible link between persistence and cell surface properties. Further investigation into the relevance of this correlation could lead to a better understanding of the persistence phenomenon in *P. aeruginosa* and to improved treatment procedures in the combat against chronic infections caused by this pathogen.

Keywords phenotypic antibiotics tolerance, persisters, LPS, lipopolysaccharides

Effect of antifungal agents on Non-*Candida albicans Candida* species enzymes secretion

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The infective ability of *Candida* species depends on specific virulence mechanisms that confer the ability to colonize host surfaces, to invade deeper host tissue or to evade host defences. During the pathogenic process many virulence attributes may be involved including, production of extracellular proteases and haemolytic activity. Nevertheless, *in vitro* studies have indicated that antifungal agents could be able to influence the enzymatic activity of *Candida* species. Therefore, the purpose of this work was to investigate the action of antifungals on proteinase and haemolytic activity of Candida species.

This study was conducted with *C. albicans* (1), *C. glabrata* (4), *C. parapsilosis* (5) and *C. tropicalis* (6) recovered from different body sites (blood, oral, vaginal and urinary tract). Four reference strains of *C. albicans* ATCC 90028, *C. glabrata* ATCC 2001, *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 750 were also examined. The susceptibility to fluconazole and amphotericin B was determined by the microdilution test in order to allow the determination of the minimal inhibitory concentrations (MIC) and the maximum antifungal concentration (MAC). Then, the proteinase and hemolytic activity was determined for yeasts grown at MIC and MAC.

It was observed that all *Candida* species assayed were sensible to both antifungal agents. Concerning the antifungal effect on enzymatic activity of Candida species, *C. parapsilosis* from candiduria presented a decreased proteinase and haemolysin activity for both MIC and MAC of both antifungal agents. Moreover, the other species presented differences in terms of production of proteinase and haemolysin at MIC and MAC. *Candida albicans* reference strain presented lower proteinase activity at MIC of fluconazole (46.7%) but presented higher activity for MAC (61.9%) in comparison to the control (60%). Furthermore, regarding haemolysin activity there were isolates that expressed high levels of enzymes in the presence of both antifungals such as: *C. glabrata* from urine and from vaginal tract; and *C. tropicalis* from urine. Conversely, some clinical isolates, presented low levels of enzymatic activity after contact with the antifungal agents, such as: *C. albicans* (oral isolate); *C. glabrata* (oral isolate and vaginal isolate); *C. parapsilosis* (from urine) and also *all C. tropicalis* except one urinary isolate.

It was possible to conclude that the proteinase and haemolysin activities were strain and species dependent and no correlation was found among activity profile and the site of isolation. Moreover, fluconazole and amphotericin B were able to influence the tested *Candida* species enzymatic activity.

Keywords: Candida; Fluconazole; Amphotericin B; Proteinase; Haemolytic activity

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Effect of chitosan, nisin and storage time on the growth of *Listeria innocua* and *Shewanella putrefaciens* in fish homogenates

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Fish and other fishery products are highly perishable food. The process of degradation starts immediately after the fish's slaughter and it often develops more quickly than in other meats. Therefore, the use of a preservation method is essential when it comes to the mentioned products. The use of natural antimicrobials together with other preservation measures has proved to be a good option to extend the shelf life of this kind of food.

The aim of this study was to evaluate the effect of chitosan, nisin and storage time on the inhibition of *Listeria innocua* and *Shewanella putrefaciens* growth in fish homogenates. For such purpose a factorial design in three blocks was performed, on which the concentration of chitosan and nisin, and the storage time were the factors studied. The selected levels were: chitosan (0, 2000 ppm), nisin (0, 5000 ppm) and storage time (0, 48 hours). A central point was included (chitosan 1000 ppm, nisin 2500 ppm, storage time 24 hours). *L. innocua* was selected as a representative microorganism of Gram-positive flora and as an alternative to the pathogen *Listeria monocytogenes*. *S. putrefaciens*, which is one of the typical spoilage microorganism of fishery products when they are stored in cold and aerobic conditions, was selected as the Gram-negative bacterium representative.

Fish homogenate was prepared processing Argentine hake fillets (*Merluccius hubbsi*) and distilled water in a ratio of 1:1; pH was adjusted to 5.5 using citric acid 10%m/m and homogenate aliquots were put into screw-cap flasks. They were sterilized for 15 minutes at 100ºC. Preservatives were added and then microorganisms were inoculated reaching a level of 105 CFU g-1. The microorganisms had been previously incubated in Mueller-Hinton broth, overnight at 30ºC. The inoculated homogenates were stored at 30ºC for 48 h. *S. putrefaciens* and *L. innocua* populations were enumerated by pour-plating in TSA agar and Mueller-Hinton agar, respectively. The plates were incubated for 48 hours at 30ºC.

As it was expected, in all cases, time exerted a significant effect on the growth of both microorganisms. When antimicrobials were added, counts were significantly reduced ($P < 0.05$). A significant interaction ($P < 0.05$) between antimicrobials and time was noted; however, this effect depended on the microorganism being tested. In the case of *S. putrefaciens*, both preservatives reduced growth during storage; on the other hand, the development of *L. innocua* was reduced by chitosan during storage, but it increased in the presence of nisin. There was no significant interaction between the two preservatives. However, different trends in respect of time are noted. At time zero, nisin alone or together with chitosan reduced the counts of both microorganisms between 2 and 3 log cycles. After 48 hours of storage, both antimicrobials showed an additive effect reducing the development of both microorganisms between 4 and 5 log cycles.

To sum up, nisin and chitosan were able to inhibit the development of *S. putrefaciens* and *L. innocua* in fish homogenate at pH 5.5. It is clear that nisin controlled the growth of *S. putrefaciens*, Gram-negative bacterium. Although the interaction was not significant, the joint use of the studied antimicrobials could be promising, since the nisin´s effects on counts are shown immediately after its application, while chitosan´s action can be noticed throughout the storage.

Keywords ; nisin, chitosan

Effect of penicillins on the acidification of yogurt made from ewe's milk during the storage

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The effects of three penicillins (penicillin G, ampicillin and amoxicillin), at concentrations close to the Maximum Residue Limits (MLRs) regulated by the European Union as safe for consumers, have been studied in yoghurts made from ewe's milk during the storage in order to know if these concentrations were also apt for the correct development of the fermentation processes. Firstly, yoghurts were fortified with three different concentrations: 2, 4 (MRL) and 6 μg/kg of each antibiotic and the acidification and microbial evolution were evaluated until 21 days of refrigeration storage. Results showed that concentrations of some of the penicillins, equal or inferior to MRL, could inhibit the normal growth of lactic acid bacteria and provoke some alterations in the acidity parameters by cusing damage on the production of the isomer L(+) lactic acid, wich is easier to assimilate by consumers.

Keywords: yogurt; ewe milk; penicillins; acidification; microbial growth

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Effect of treatment with organic acids on antibiotic resistance patterns of *Escherichia coli* isolates from poultry during refrigerated storage

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Background: Concerns about bacterial drug resistance have been growing for a number of years and have been raised at both national and international levels. Recent scientific evidence suggests that the selective pressure exerted by the use of biocides, including compounds widely used in the food industry, could contribute to the expression and dissemination of antibiotic resistance mechanisms. Chemical decontamination of red meat and poultry carcasses is prohibited in the European Union on the basis of the Precautionary Principle. The need to continue to collect new data so that the development of antimicrobial resistance by the use of decontaminants could be fully assessed has been highlighted by the European Union Agriculture and Fisheries Council. The aim of this study was to determine whether the decontamination of poultry legs with organic acids (citric acid and ascorbic acid) influences the prevalence of resistance to antimicrobial drugs in *Escherichia coli* populations during refrigerated storage.

Methods: Chicken legs (30) collected from a local poultry processing plant immediately after evisceration were dipped for 15 min into 500 mL of sterile solutions of 2% (w/v) citric acid (Panreac, Barcelona, Spain; 10 samples), 2% (w/v) ascorbic acid (Sigma-Aldrich Química S.A., Madrid, Spain; 10 samples) or were not treated (control; 10 samples). All samples were evaluated for microbiological quality after 0 (immediately after the dipping treatment had been completed) and 6 days of storage at 7±1º C. For the recuperation of bacteria, 5 g of skin were homogenised in 45 mL of buffered peptone water (Oxoid Ltd., Hampshire, United Kingdom), and adequate dilutions were pour plated in VRBA (Oxoid Ltd.) with overlay. Plates were incubated at 44±1º C for 24 h. Isolates were preliminarily investigated by their colony and cell morphology, Gram stain, oxidase and catalase activities. Presumptive *E. coli* strains were confirmed by the *E. coli* test (Liofilchem s.r.l., Teramo, Italy) identification system. A total of 150 strains (25 for each treatment and sampling day) were used for antimicrobial susceptibility testing. The isolates were screened for susceptibility to a panel of 12 antibiotics on Mueller-Hinton agar (Oxoid Ltd.) by a disc diffusion method (CLSI). The prevalence of resistant strains and multi-resistance patterns were compared by means of the chi-square and the two-tailed Fisher's exact test. The tests were carried out using the Statistica® 6.0 software package (Statsoft Ltd., Tulsa, Ok, USA).

Results: Four (2.67%) isolates were pan-susceptible, 10 (6.67%) resistant to one compound, and 136 (90.67%) showed multi-resistance (resistance to two or more antimicrobials). The average number of resistances per strain was 4.47. The highest average prevalence of resistance was observed for tetracycline (84% of strains tested), amoxicillin-clavulanic acid (80.67%) and nalidixic acid (77.33%), followed by sulfamethoxazole-trimethoprim (64%) and ciprofloxacin (60%). The treated samples showed higher ($P<0.05$) percentages of resistant strains than the control samples in the case of amoxicillin-clavulanic acid (legs treated with ascorbic acid and tested on day 6 of storage), sulfamethoxazole-trimethoprim, ciprofloxacin (citric acid and ascorbic acid; day 6) and tetracycline (citric acid; day 6).

Conclusion: Our results suggest that the treatment with organic acids could, in certain conditions, increase the prevalence of resistance to antibiotics in bacterial populations on poultry, making these treatments potentially dangerous for consumers. Further studies in order to confirm these findings in detail should be performed previously to the authorization of organic acids as poultry decontaminants in the European Union.

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Keywords: antimicrobial treatments; organic acids; antibacterial resistance; *Escherichia coli*; poultry.

Effect of $ZnCl_2$ on growth and survival of table olive related yeasts

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Zinc (Zn) is an essential microelement which is involved in numerous enzymes related to the metabolism of carbohydrates, proteins and fats. However, there is usually a deficient Zn daily intake with respect to recommend by experts (15 mg/day). Preliminary studies carried out in our laboratory on the effect of the fortification of table olives with Zn, showed a possible inhibitory effect of this microelement on the yeast micro-flora associated with seasoned cracked table olives. For this reason, a detailed study of the effect of $ZnCl_2$ on the growth and survival of diverse yeasts isolated from table olives was performed. Growth experiments were conducted in a Bioscreen C equipment using laboratory medium supplemented with progressive amounts of $ZnCl_2$. It was observed a great diversity among the responses of yeasts, although all of them were markedly affected by the presence of this compound. One of the most resistant yeasts was *Saccharomyces cerevisiae*, which was in agreement with the presence of exclusively this yeast in packed olives with $ZnCl_2$. With respect to the survival (time to killing curves), it was also observed diverse behaviour among yeasts. In some cases, there was a further recuperation of the population after an initial period of inhibition. Results are promising with respect to the possible use of this microelement to fortify table olives and simultaneously improving the stability of the final product enlarging its shelf life.

Keywords: zinc; yeast, fortified table olives; inhibition, packing.

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Effectiveness of a novel postmilking teat dipping based on terpinen-4-ol compared to a customary product in reducing the incidence of intramammary infections in dairy sheep

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Introduction. Till today, mastitis is the most common and expensive disease of dairy herds and therefore, the single largest cause of antibiotic usage in dairy herds. This provides favourable conditions for selection, spread and persistence of antimicrobial resistant bacteria capable of causing infections in animals and humans. The awareness of the potential problems correlated to antimicrobial resistance has promoted worldwide different strategies to try to limit the antibiotic selective pressure. In this light, the research of new effective non-antibiotic agents seems to be a very promising attraction. Postmilking teat dipping, containing a biocide solution, practice is considered advantageous to prevent mastitis in dairy animals but, while its effectiveness to prevent bovine mastitis is well documented, no information is available for dairy sheep. In the framework of TRUEFOOD project, terpinen-4-ol (T-4-ol), the major antimicrobial component of *Melaleuca alternifolia* essential oil was investigated for its applicability in a novel postmilking teat dipping for mastitis prevention in different lactating animal species (European Patent application, No. 08425431.7).
Aim of this study was to evaluate the effectiveness of terpinen-4-ol as active ingredient of a postmilking teat dipping preparation, in reducing the number of new intramammary infections (IMI) in comparison with a traditional postmilking teat dipping at 0.5% of chlorexidine, when applied in dairy sheep.

Materials and methods. The trial was carried out in a well-managed dairy sheep herd from February to June 2010. Thirty Sarda ewes were selected and divided into experimental and control group, balanced for age, production and health status. After each milking the experimental group was treated dipping all teats in the T-4-ol product while the control group was treated with the 0.5% chlorexidine dipping., Half-udder milk was collected bi-monthly from each animal after thorough teat disinfection and tested for bacteriological analysis and somatic cell count. To evaluate criteria for diagnosing infection, every single half udder was considered as an independent sample. An IMI was diagnosed when the same bacterial specie was isolated consecutively from bi-monthly samples or from clinical half-udder milk samples or when half-udder milk somatic cell content (CCS) was >268000/ml (physiological threshold in sheep milk determined consecutively from the samples taken bi-monthly), or for a bacterial isolation associated with a CCS >268000 cell/ml, for the same sample. Data analysis was conducted following the guidelines recommended by the National Mastitis Council, computing a 95% one-side lower limit (LLCI) confidence interval for the difference between proportions using the normal approximation and the critical one-tailed Z of 1.645.

Results. Data showed 15 and 13 new IMI of 25 and 29 eligible half-udders in control and experimental group respectively. The fraction of new IMI was 0.60 in control and 0.34 in experimental; the pooled infection proportion was 0.46. The percentage reduction in new IMI was 42.53 %.LLCI value was 0.031, which is positive and suggests that the terpin-4-ol post-dipping product is more effective than 0.5% chlorexidine dipping in reducing the incidence of new infections by at least 3.1%.

Conclusions. T-4-ol postmilking teat dipping resulted a good alternative to a post dipping containing chorexidine when applied in dairy sheep for mastitis control. This result must be considered also in the light of the growing bacterial resistance to chlorexidine, one of the most common biocides used in postmilking teat dipping formulation, while very low frequencies of mutation for resistance were obtained by tests on T-4-ol.

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Keywords: Terpin-4-ol, post-dipping, mastitis, sheep

Emergence of Multi-drug-resistant *Salmonella* Concord infections in Belgium linked with children adopted from Ethiopia, 2004-2009

Raymond Vanhoof, Cécile Boland, Olivier Vandenberg, Frédéric Fux, Maria Naranjo, Jean-Marc Collard and Sophie Bertrand

Antibiotic treatment is not required in *Salmonella enterica* gastroenteritis but is essential in enteric fever, invasive salmonellosis or in immunocompromised patients. Although fluoroquinolones and third-generation cephalosporins (CEF3) are the drugs of choice to treat invasive *Salmonella*, resistance to these antibiotics is increasing worldwide.

Since 2004, an increasing number of infections caused by *Salmonella* serovar Concord (a rare serotype) were reported in Belgium among children adopted from Ethiopia. Between 2004 and 2009, 38 cases of laboratory confirmed *Salmonella* Concord were reported by the National Reference Centre for *Salmonella*. With the exception of 3 adults, only children were affected. The mean age of these children was 0.9 year (range 0.1 – 3.2 y). The Ethiopian adoption status was known for 29 children. Two cases were due to contact with an adopted child and 1 case concerned a traveller to Ethiopia. For 6 cases, a link with Ethiopia could not be found. In six cases, the children carried the *Salmonella* Concord varying from 1.1 to 5.2 months.

Susceptibility to 14 compounds was tested by disk diffusion according to CLSI. Resistance to ampicillin (AM), Cefotaxime (CX), Streptomycin (SM), and Sulfonamides (SU) was found in 97.0 % of the isolates. Resistance to Gentamicin (GM), Trimethoprim (TR) and co-Trimoxazole (S/T) was present in 93.9 % of the isolates. Resistance rates to the other compounds were as follows: 81.8 % to Chloramphenicol (CL), 78.8 % to Tetracycline (TE), 15.2 % to Nalidixic Acid (NA), 6.1 % to amoxicillin/clavulanic acid (A/C) and 3.0 % to Kanamycin (KM) and Spectinomycin (SC). Furthermore, intermediate resistance to NA was found in 24.2 % of the isolates while intermediate resistance to KM, SM and TR was found in 3.0 % of the isolates. One isolate showed only intermediate resistance to SM. Non susceptibility to Ciprofloxacin was not found. The vast majority of the isolates presented a multi-drug-resistant profile to antimicrobial agents. Multi-drug-resistance to 9 and more compounds was present in 72.7 % of the isolates. The most prevalent multi-drug-resistant profile was resistance to AM-CX-TE-TR-CL-GM-SM-SU-S/T. Isolates were subtyped by pulse-field gel electrophoresis and specific antimicrobial resistance genes were characterized.

This study provides useful information for parents adopting children from Ethiopia and for their family practitioner. It also highlights the importance of prophylactic measures and appropriate hygiene both at school and in the social environment of the child to prevent further dissemination.

Keywords *Salmonella* Concord; multi-drug-resistance; adoption; carriage

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Emergence of NDM-1 producing multidrug-resistant *Acinetobacter baumannii* in a German hospital

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Introduction. Emergence and dissemination of multidrug-resistant *Acinetobacter baumannii* are of special concern because of limited therapeutic options and increased mortality. In many cases colistin is the only antimicrobial substance for an adequate treatment. The carbapenem resistance in *A. baumannii* is mainly due to production of different carbapenemases like diverse OXA-enzymes or metallo-beta-lactamases. Most prevalent are the class D types OXA-23, OXA-58 and OXA-40-related beta-lactamases. These bla_{OXA} genes are frequently located on transferable plasmids. However, the insertion sequence *IS*Aba1 can also cause hyperpoduction of the intrinsic OXA-51-like carbapenemase in *A. baumannii*. Moreover VIM-2 and IMP-1 metallo-beta-lactamases were described in single isolates and outbreak strains. In Germany outbreaks often occur in the summer months when travellers that were hospitalized abroad are repatriated. Here we report on retrospective molecular-epidemiological analysis of multidrug-resistant *A. baumannii* isolated in a German hospital in 2007.

Methods. In 2007 ten multidrug-resistant *A. baumannii* were isolated on a surgical ICU of a university hospital. The strains were isolated from tracheal secretion (n=2), nasal swab (n=1), throat swab (n=1), inguinal swab (n=1) and fecal swabs (n=4). Most patients were only colonized with *A. baumannii*. The supposed index patient was hospitalized previously in Egypt. Antimicrobial susceptibilities to different antibiotics were determined by broth microdilution and Etest. Occurrence of β-lactamases was detected by PCR amplification and sequencing of relevant resistance genes. For selected isolates transfer of resistance was tested by plasmid isolation and transformation into an *A. baumannii* ATCC 17978 recipient. Molecular typing by PFGE and sequence-based multiplex PCR to identify isolates belonging to members of the European clonal complexes I-III were performed.

Results. All *A. baumannii* isolates were resistant to fluoroquinolones, aminoglycosides and beta-lactams including carbapenems. PCR and sequence analysis revealed the occurrence of OXA-23 carbapenemase and TEM-1 beta-lactamase in nine isolates. For the remaining isolate Etest for metallo-beta-lactamase was positive. The new NDM-1 enzyme was identified in this strain. Macrorestriction analysis by PFGE showed nearly identical patterns for eight strains. One OXA-23 producer and the bla_{NDM-1} positive isolate were not related to these isolates. Multiplex-typing PCR revealed that the eight clonal-related isolates belong to the European clonal complex II. The single OXA-23 producing isolate was related to European clonal complex I. The strain harbouring bla_{NDM-1} was not related to one of the three EU-clones. It contained the typical marker genes *csuE* and bla_{OXA-66} but no *ompA*. Transformation of resistance plasmids containing bla_{OXA-23} were successful in the strains related to clone EUI and EUII but failed for the NDM-1 producer.

Conclusion. Infections and outbreaks with multidrug-resistant *A. baumannii* are observed every year in many German hospitals. The results of this study provide evidence for a clonal dissemination of an OXA-23 producing strain within the hospital in 2007. This strain was probably introduced by a patient from Egypt. After initiation of strict hygiene control measures the outbreak was under control and no more cases occurred. Two *A. baumannii* isolated within the of the outbreak period were not related to the outbreak strain. Interestingly, one isolate harboured the gene coding for the new metallo-beta-lactamase NDM-1. This plasmid-mediated enzyme was first identified in *K. pneumoniae* from a Swedish patient after his return from New Delhi (India) in 2008. Recently NDM-1 was also found in *E. coli, K. pneumoniae* and *E. cloacae* in the USA. To the best of our knowledge this is the first description of NDM-1 in *A. baumannii.* Although transferability could not be proved yet a conjugative transfer of bla_{NDM-1} containing plasmids from Enterobacteriaceae in *A. baumannii* is certainly possible. NDM-1 producing bacteria have the "superbug potential". Therefore there is an urgent need for surveillance of all multidrug-resistant strains inside and outside the hospitals to prevent further dissemination.

Keywords: NDM-1 metallo-beta-lactamase; OXA carbapenemase

Environmental and demographic risk factors associated with prevalence of Cryptosporidium infection in the Alice rural settlements of the Eastern Cape Province of South Africa: a pilot study

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Cryptosporidium is a well established cause of diarrhoea among immunocompromised patients worldwide. *Cryptosporidium* diseases attributable to environmental contamination occur more commonly in other regions of South Africa. However, no information exists regarding infection in the Eastern Cape Province, which is predominantly rural with poor service delivery. We therefore undertook this study to identify risk factors and prevalence of cryptosporidiosis in HIV-positive and HIV-negative diarrhoea patients in Alice rural settlement. A total of 180 stool specimens comprising 35 HIV positive-diarrhoea, 125 HIV negative-diarrhoea patients and 20 apparently healthy subjects were screened for cryptosporidiosis using an ELISA based approach. All diarrhoea positive patients were interviewed to record socio-demographic information, water supply and animal contact. Data were analysed using Pearson's $\chi 2$ and Fisher's exact test to assess the univariate association between *Cryptosporidium* infection and the possible risk factors. Of the 180 subjects screened for cryptosporidial infection, *Cryptosporidium* antigen was detected in 122 giving an overall prevalence of 67.8%. The ages between 31-43 (mean age 36.5 yr) and 70-82 (mean age 75.8 yr) had a higher prevalence (100%) of the antigen than 18-30 (mean age 23.2 yr) and 83-95 (mean age 88.8 yr) (50.0%) in HIV-positive diarrhoea patients ($P > 0.05$; OR = 0.75, 95% Cl: 0.34-1.62). In HIV-negative diarrhoea patients, prevalence was highest in the 18-30 (mean age 23.2 yr) (87.5%) and least (35.7%) in those aged 83-95 (mean age 88.8 yr) ($P > 0.05$; OR = 0.92, 95% Cl: 0.68-1.23). *Cryptosporidium* antigen was higher in females than in males. Of 115 female (mean age 46.7yr) who participated in the study, the antigen was detected in 90 (78.2%) against 32 (71.1%) of 45 males (mean age 42.6yr). None of the 20 apparently healthy control subjects was found to be infected with *Cryptosporidium.* A significantly higher ($P < 0.05$) prevalence of antigen was observed in HIV-negative diarrhoea than HIV-positive patients considering contact with farm animals as a risk factor. Prevalence peak (85.7%) was detected in low income HIV-positive diarrhoea patients than high income (32%) of same category of patients. Differences found in prevalence rates due to water source, suggest that the high infection rates of specific groups are associated with their exposure to the contaminated water supply. The results indicate that *Cryptosporidium* infection is highly prevalent in adult faecal specimens in the Nkonkobe Municipality, an indication of active infection that is likely to emerge as a major human pathogen in this locality due to socioeconomic changes which favour transmission.

Key Words: Prevalence; *Cryptosporidium*; antigenemia; diarrhoea; risk factors; HIV; South Africa.

Epidemiology of multi-drug resistant Acinetobacter baumannii at a Teaching Hospital in Italy

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Introduction: Multi-drug resistant Acinetobacter baumannii (MDR-Ab) is problematic in tertiary-care hospitals. In the last years, the clinical relevance of MDR-Ab in the community setting has increased to reach a pivotal role. The aim of this study was to analyze the risk factors associated to infection / colonization by MDR-Ab, to verify the nosocomial or communitarian origin of these different isolates, and to genetically characterise the different strains of MDR-Ab.

Materials and Methods: From July 2008 a MDR surveillance system has been implemented by the Hospital Hygiene Service in collaboration with the Microbiology Section of the Laboratory Analysis of the AOU Ospedali Riuniti of Ancona, Italy. Thanks to this system, it was possible to detect isolates of MDR-Ab circulating in the hospital setting. All the first isolates of MDR-Ab were stored from January to June 2010. Individual cases have been analysed through review of medical and laboratory records. The data acquired for the positive patients and risk factors for MDR-Ab infection/colonization included: demographic data, source, type of admission, comorbidities, presence, type and duration of invasive devices, antimicrobial use before the first isolation of MDR-Ab; moreover, the presence of sepsis, ventilator-associated pneumonia, catheter-associated urinary tract infections, and surgical wound infections was assessed. Three populations of patients were identified: MDR-Ab positive patients within 48 hours from admission to the ward; MDR-Ab negative patients at screening, or with previous negative microbiological examination that have become positive to MDR-Ab; patients who have not been screened within 48 hours from admission or do not have a previous microbiological examination, but subsequently have a positive isolation for MDR-Ab.

To determine the genetic relatedness of MDR-Ab isolates described in this study, we carried out pulsed-field gel electrophoresis (PFGE).

Results and discussion: This study included 60 patients with MDR-Ab positive isolates. Three populations of patients have been identified: 19 patients were screened on admission, or within 48 hours from admission to the ward (31.66%), and 11 were positive (18.33%); 5 of these patients came from home (8.33%), 6 patients were transferred from another hospital or tertiary care. We also have identified 41 patients who do not have a screening at admission, but alater submitted an isolation positive for MDR-Ab (68.33%). The Pulsed Field Gel Elecrophoresis (PFGE) has allowed to establish a close relationship for many clinical isolates, (hospital *A.baumannii* strains vs. community strains).

Conclusions: Our results highlight that the majority of infections have been contracted in the hospital setting. However, six isolates belonging to patients coming from nursing homes, and five isolates belonging to patients without a previous history of admission to an hospital or a community setting put into evidence the possible role of the community in the circulation and epidemiology of MDR-Ab.

Keywords: Acinetobacter baumannii, multidrug resistance, molecular epidemiology

ESBL- producing in Enterobacteriaceae the Northern Portugal – Antimicrobial Susceptibility and Molecular Epidemiology

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Extended-spectrum β-lactamases (ESBLs) prevalence was studied in the north of Portugal, among 193 clinical isolates belonging to citizens in a district in the boundaries between this country and Spain. It were recovered some members of *Enterobacteriaceae* family, producing ESBL enzymes, including *Escherichia coli* (67.9%), *Klebsiella pneumoniae* (30.6%), *Klebsiella oxytoca* (0.5%), *Enterobacter aerogenes* (0.5%) and *Citrobacter freundii* (0.5%). The β-lactamases genes, bla_{TEM}, bla_{SHV} and bla_{CTX-M} were screened by Polymerase Chain Reaction (PCR) and sequencing approaches. TEM enzymes were among the most prevalent types (40.9%) followed by CTX-M (37.3%) and then SHV (23.3%) types. Almost all ESBL-producing isolates (98.9%) were resistant to the fourth-generation cephalosporin Cefepime. Of the 193 isolates 157 (81.3%) present transferable plasmids. Clonally studies were performed by PCR for the Enterobacterial repetitive intragenic consensus (ERIC) sequences. This study reports a high diversity of genetic patterns, A to J for *E. coli* isolates and *K* to O for *K. pneumoniae* strains.

Keywords Enterobacteriaceae; ESBL

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Escherichia Coli Tetracycline R-Plasmids and Tetracycline Residues in Chickens

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A total of 57 *Escherichia coli* was isolated from chicken intestines. The isolates were challenged against 15 antibiotics. All strains were resistant at least one antibiotic and 50% were multiple resistant to several antibiotics. Most of the isolates were resistant to tetracycline (97.9%). DNA analysis for the most common tetracycline resistant (Tc^r) genes, *tet* A and *tet* B, revealed that the majority of isolates contained *tet* A (82.6%) and (73.9%) contained *tet* B. Among the isolates, 56.5% carried the two Tc^r genes. Chicken livers and kidneys were tested for the presence of tetracycline derivatives using high performance liquid chromatography-tandem quadrupole mass spectrometry (HPLC-MS-MS). All the collected samples were tested for chlortetracycline (CTC), oxytetracycline (OTC), doxycycline (DC). The samples contained tetracycline's residues at 0.13-0.65 pg/µl levels. The concentration of the detected antibiotics was below the maximum residue limits (MRL).

Evaluation of the susceptibility of isolates of *Enterobacteriaceae* to quinolones and fluoroquinolones isolated from some laboratories of medical analyse in the region of Bejaia (Algeria)

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Purpose: The aim of the present study was to evaluate the sensitivity to quinolones of *Enterobacteriaceae* strains isolated in the community in the region of Bejaia (Algeria) and to determinate risk factors associated with colonization strains resistant to quinolones.

Methods: Strains of *Enterobacteriaceae* are collected from four laboratories of medical analyses in the region of Bejaia (Algeria) in 2008 and 2010. These strains are tested toward nalidixic acid, fluoroquinolones and also other antibiotic families. MIC of ciprofloxacin is performed to quinolone resistant strains and some of these strains are analysed by PCR of *qnr* gene and *bla* $_{CTX-M}$ gene.

Results: The results reported a rapid evaluation for nalidixic acid resistance 14.73% in 2008 and 25.87% in 2010. The statistical analysis showed that age and sex were risk factors associated with colonization by *Enterobacteriaceae* strains resistant to quinolones.

The determination of ciprofloxacin's MIC reported high levels of ciprofloxacin resistance and the PCR analyses showed that 3 strains were *qnr*-B1 positive (2 strains of *E. cloacae* and 1 strain of *E. coli*) among these strains, two were CTX-M ESBL producing.

Conclusion: Increasing importance quinolone resistance among *Enterobacteriaceae* in our region gives causes for great concern.

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Factors affecting the sensitivity of Gram-negative bacteria to lysozyme during freeze-thawing and mild heating process

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Hen egg lysozyme catalyses the hydrolysis of β-1,4 linkages between *N*-acetyl muramic acid and *N*-acetyl glucosamine in peptidoglycan layers of bacterial cell wall and shows a lytic activity to some Gram-positive bacteria. So it has been applied as an additive to extend the shelf life of foods with some cooperative additives. Gram-negative bacteria, however, are not sensitive to lysozyme because of their outer membrane. It is known that some physical and chemical stresses can give damage to the outer membrane and alter its permeability to antimicrobial agents. The damaged or injured bacterial cells would be more sensitive to the agents. Thus hurdle technology has been applied to preservation of foods, especially minimally-processed-foods and ready-to-eat foods, using the physical or chemical stress as a hurdle. In this report, the sensitivity of Gram-negative bacteria to lysozyme under sublethal mild heating and freeze-thawing conditions and factors influencing on the lysozyme sensitivity were investigated.

Treatment with 1mg/ml of lysozyme (Eisai) under heating at 50°C for 30 min in PBS (pH 6.2) significantly decreased plate counts on Tryptone soya agar (TSA, Oxoid) of *Micrococcus luteus* NBRC 3333^T, *Bacillus subtilis* NBRC 13719^T and *Listeria monocytogenes* IID 581, and also affected to *Escherichia coli* NBRC 3301, *E. coli* O157: H7 RIMD 0509939, and *Salmonella* Enteritidis NBRC 3313, while the Gram-negative bacteria were insensitive to lysozyme at 35°C. Also as a result of freeze-thawing treatment in the presence of lysozyme at -18°C or -69°C for 7 days in PBS, viable counts of *E. coli* and *Sal.* Enteritidis were reduced.

The physicochemical characteristics of *E. coli* NBRC 3301 as a typical Gram-negative bacterium were investigated during the mild heating and freeze-thawing treatments. Incorporation of *N*-phenyl-1-naphtylamine into *E. coli* cells just after heating at 50°C or freezing treatment at -18°C and -69°C increased from before the treatment. It was confirmed by SDS-PAGE that lysozyme significantly adhered on the bacterial cell during heating and freeze-thawing processes. Thus mild heating and freeze-thawing processes could cause injury of the outer membrane of *E. coli* cells. Concurrently, coexisting lysozyme would adhere on the cell surface, stick into the outer membrane and permeate to the target site. On the other hand, after these stresses were removed, *E. coli* cells could immediately recover from injured state and turn insensitive to lysozyme. The bactericidal effect of lysozyme was not observed for *E. coli* cells that previously heated and then cooled. Similarly, *E. coli* became sensitive to lysozyme present at the moment of thawing treatment, but showed less sensitivity to lysozyme added after thawing.

Heating or freeze-thawing process as a stress could cause injury of the cell membranes of *E. coli* to increase its sensitivity to lysozyme. This phenomenon might be influenced by food constituents, protecting the cells from injury, such as sugars and cations. Some sugars show cryoprotective effect to live cells and proteins. Influence of sugars on the sensitivity of Gram-negative bacteria to lysozyme during freeze-thawing and mild heating process was investigated. Under freeze-thawing condition, lysozyme sensitivity, NPN incorporation, and released intracellular substances of *E. coli* were reduced in the presence of 200-300 mM sucrose. Sucrose in the system might protect the outer and cytoplasmic membranes of *E. coli* cells from the freeze-injury, and as the results, the sensitivity of *E. coli* to lysozyme should not so increased in the presence of sucrose during freezing treatment. Under the mild sublethal heating condition at 50°C, however, the protective effect of sucrose for *E. coli* cells was not observed and lysozyme sensitivity and injured cell membranes of *E. coli* were increased with sucrose concentration. Thus influence of sugars on the lysozyme sensitivity of *E. coli* during freeze-thawing process was different from that during mild heating. Application of lysozyme may be effective for the preservation of insufficiently heated foods or frozen-chilled foods, in consideration of influence of processing and food constituents on the antibacterial efficacy.

Keywords; lysozyme, Gram-negative bacteria, stress injury, freeze-thawing, mild heating

Farm animals as a reservoir and source of environmental contamination by staphylococci and enterococci with dangerous types of resistance

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The purpose of this study was to investigate the prevalence of erythromycin resistant staphylococci and enterococci in selected farm animals. A total of 600 pig and dairy cattle rectal swabs and 300 turkey cloacal swabs were sampled between March 2009 and May 2010 from 18 different farms (6 farms per animal species) in the Czech Republic. A total of 120 and 41 erythromycin resistant isolates of *Staphylococcus* spp. and *Enterococcus* spp., respectively, originated predominantly from turkeys (75.8% staphylococci and 92.7% enterococci), followed by pigs (10.8% staphylococci and 4.9% enterococci) and dairy cattles (13.4% staphylococci and 2.4%). Determination of resistance to erythromycin as well as cross resistance to macrolides, lincosamides and streptogramin B (MLS_B resistance) were performed according to the recent CLSI standards. MLS_B resistance was confirmed phenotypically in 90 isolates (65 isolates of staphylococci and 25 isolates of enterococci). Among these, 86 (95.5%) were from turkeys (63 staphylococci and 23 enterococci), three (3.3%) from pigs (one *Staphylococcus* spp. and two enterococci) and one from dairy cattle (one *Staphylococcus* spp.). MLS_B resistance was constitutive in all except for five isolates (4.2%; all of staphylococci) showing inducible MLS_B phenotype. By PCR, the isolates were examined for the presence of *erm*A, *erm*B, *erm*C, *msr*A and *mph*C genes. Among staphylococci, 0.8% of isolates harboured the *erm*A gene, 9.2% *erm*B, 47.5% *erm*C, 45% *msr*A and 35% *mph*C gene. Furthermore, 9.8% of enterococci harboured the *erm*A gene, 60.9% *erm*B, and 43.9% *mrs*A gene. None of the erythromycin resistant enterococci had the *erm*C and *mph*C genes. In three MLS_B positive staphylococci, the methicillin resistance was confirmed by a *mec*A specific PCR. In addition, MLS_B positive staphylococci (n = 5) and enterococci (n = 3) were shown to have inducible resistence to telithromycin (ketolides). This study revealed that turkeys may be an important reservoir of erythromycin and MLS_B resistant gram positive cocci.

Keywords erythromycin; clindamycin; resistance; MLS_B; erm; food safety

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Frequency and characterization of class 1 integrons from commensal *Escherichia coli* strains from pigs and broilers lacking the *qacEΔ1- sul-1*on the 3`conserved region

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Introduction: The discovery of plasmids and transposon was the begining of the understanding of the genemobility between bacteria of the same and different specie. In the eightees the discovery of integrons by Stokes and Hall was done through the systemic analysis of transposons and resistant plasmids which contained a large number of interrelated genes. Integrons are defined as genetic configuration shaving a specific recombination site in the 5‘ conserved region (CS), followed by a variable region where are allocated the genes cassettes captured. In the 3´CS there are usually genes conferring resistance to amonium quaternary (*qacEΔ1)* and sulphonamides(*sul1).*Another sulphonamide resistance gene(*sul3),* initially detected in a conjugative plasmid identified in *Escherichia coli* isolated from pigs, was lately also associated with class 1 integrons (Antunes et al.), after being identified in *Salmonella* and *E coli* strains. In both bacteria the integrons were lacking genes *qac*Δ*E1* and *sul1,* being replaced by the genes *qacH+tnP440+sul3*.

Aim: To study the frequency and evolution of this unusual integrons class 1 among *E. Coli* strains from food producing animals.

Methods and Results: A Spanish collection of 393 *E. Coli* strains obtained in two periods (1998/99 and 2006) from faecal samples from pigs (PO-98/99 (N=100); PO-06 (N=97)) and broilers (AV-98/99 (N=100); AV-06 (N=96)) was previously studied for class 1 integron, detecting a high frecuency in both species being more frequent in pigs (105= 60(PO-98/99) + 45(PO=06)) than in broilers (81= 40(AV-98/99) + 41(AV-06)), decreasing in pigs and remainingstable in broilers(Marchant et al.). To study the variable region, there were used the primers described by Levesque and Roy. In those strains were the variable region could not be amplified there were considered the three integron configurations detected by Antunes et al., as a model to determine the configuration. In first place there were amplyfied the *cmlA* and *sul3* genes using the primers described by Saenz et al., and by Perreten and Boerling, respectively. Considering them a in genes present in these integrons (*aadA2+cmlA+aadA1+qacH-sul3*), we used the primer walking technique to link the genes contained in the integron variable region.Once obtained the diferent amplicon sizes, there were sequenced the representative onces from each group.In this study, unusual class 1 integrons were classified in two clusters; integrons with unknown 3`CS (PO-99/98(1), PO-06(3); AV-98/99(1), AV-06(5)), and integrons harbouring *qacH+tnP440+sul3*(PO-99/98(17), PO-06(12); AV-98/99(5), AV-06(10)). In the last cluster five configurations were detected; *dfrA12 + orfF + aadA2 + cmlA + aadA1 + qacH + tnP440 + sul3* (Antunes et al. type I),*sat + psp + aadA2 + cmlA + aadA1 + qacH + tnP440 + sul3* (Antunes et al. type IIII)*, dfrA12 + aadA2 + cmlA + aadA1 + qacH + tnP440 , dfrA1+aadA1+cmlA+aadA1+qacH+tnP440 + sul3 , dfra12 + orfF + aadA2 + (unknown genes)*(probably similar to Antunes type II). In relation with integrons harbouring *qacH+tnP440+sul3* in its 3`CS, there were observed a decrease in pigs (PO-98/99(41%); PO-06(27%)) because of diminishing of type I, and an increase in broilers (AV-98/99(15%); AV-06(37%) due to the rise of type III.

Conclusions:Despite of the low detection of these unusual integrons in previous studies, integrons harbourin g*qacH+tnP440+sul3* in its 3’CS, are not a new configuration, being already present in *E. Coli* strains isolated in 1998-1999. Moreover these data indicates that they are still common in pig and broiler *E. Coli* strains sugesting them as a potential reservoirs.

Keywords: *Escherichia coli*, class I integrons, sul3, qacH, tnP440, pigs, broilers.

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Frequency of thermophylic *Campylobacter* spp. on quail (*Coturnix coturnix*) samples collected in a Portuguese slaughterhouse

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Campylobacteriosis is on the top of zoonotic diseases reported in Europe and USA. *Campylobacter jejuni* and *Campylobacter coli* are the main pathogenic species causing infection after food or water ingestion, with watery diarrhea and/or hemorrhagic colitis associated with abdominal pain and fever symptoms that could persist frequently on children and adolescent, and particularly in immune-compromised patients. The main source of *Campylobacter* infection is attributed to broiler meat. At Portugal, was reported an increase of 5.3% on quail production in the last years (INE, 2010). In fact, quail meat is very appreciated by Portuguese consumers. For this reason it is important to screen the presence of *Campylobacter* spp. in quail meat as a possible source of infection to consumers. The collection of samples was made during 2009, in a Portuguese slaughter house, on different working days and for different flocks quail producers (n=9) comprehending a total of 89037 birds. For each flock, a pool of 10 intestines (caecum) was sampled, and the neck skins of 30 carcasses were collected after washing, constituting a pool sample. Two individual carcasses from each flock were taken after cooling tunnel for breasts sampling. Detection of *Campylobacter* was performed according to EN/ISO 10272-1:2006 and isolates were identified by multiplex PCR. The contamination of quail flocks/producers analysed with *Campylobacter* was very high; 78% of the samples from intestine were positive to *C. coli*. The carcasses from different flocks were 78% positive for *Campylobacter coli*. After carcass fast cooling, the frequency of detection for *C. coli* on breast carcass was again 78%. The specie *C. jejuni* was detected for only 22% of the flocks breast carcasses evaluated. The frequency of contamination with *Campylobacter* spp. make advisable a good implementation of biosecurity measures and good hygiene practice for production as well HACCP programs regarding the hazard *Campylobacter* at slaughter house level.

Keywords: *Campylobacter,* quail, frequency, safety, poultry.

Genetic characterization of vancomycin-resistant enterococci isolates from slaughtered pigs.

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Background: The increasing evolution and spread of antimicrobial drug–resistant bacteria and resistance genes seriously threaten public health; the food chain is thought to be responsible for an increasing incidence of vancomycin-resistant-enterococci in human nosocomial infections. Our aim was to study the prevalence of faecal carriage of vancomycin-resistant-enterococci (VRE) in pigs slaughtered for human consumption and study the phenotypic and genotypic characterization of antibiotic resistance mechanisms in VRE.

Methods: The presence of faecal VRE was investigated in 71 faecal samples of slaughtered swine, in a Portuguese abattoir, for human consumption.The samples were seeded in Slanetz-Bartley agar plates supplemented with 4 mg/L of vancomycin for VRE recovery and incubated 48 hours at 45°C. Colonies with typical morphology were identified by biochemical tests and specific PCR for the different enterococcal species. Antibiotic susceptibility was tested for 11 antibiotics (µg/disk): vancomycin (30), teicoplanin (30), ampicillin (10), streptomycin (300), gentamycin (120), kanamycin (120), chloramphenicol (30), tetracycline (30), erythromycin (15), quinupristin-dalfopristin (15), and ciprofloxacin, (5) by disk and agar dilution methods CLSI. Vancomycin resistance genes (*van*A, *van*B, *van*C-1, *van*C-2/3) and other resistance genes [*tet*(M), *tet*(L), *erm*(B), *aph*(3´)-IIIa, *ant*(6)-Ia, *vat*(D) and *vat*(E)] were tested by PCR, using specific primers. **Results:** Twenty-five of the 71 fecal samples analyzed showed high level of vancomycin (MIC ≥ 128 µg/ml) and teicoplanin (MIC 64 µg/ml) resistance. *Van*A containing enterococci were detected in 17 (24%) of the samples, and they were identified as *E. faecium*. Enterococci with intrinsic vancomycin resistance, *van*C1 gene, were found in 7 (10%) of the fecal samples analyzed. No van genes (*van*A, *van*B, *van*C-1, *van*C-2/3) could be identified in one of VRE detected. High percentages of antimicrobial resistance were detected to tetracycline (100%) and erythromycin (88%), and intermediate percentages of resistance were obtained to streptomycin (40%), quinupristin-dalfopristin (36%). Antimicrobial resistance percentages of 20% were detected to kanamycin and ampicillin and 12% to ciprofloxacin. None of the isolates was resistant to gentamicin or chloramphenicol. The *tet*(M) gene, related with tetracycline resistance, was found in twenty of resistant isolates, in 18 of them in association with *tet*(L) gene; five of tetracycline-resistant isolates only harbored the *tet*(L) gene. All erythromycin-resistant isolates but one contained the *erm*(B) gene. The *aph*(3`)-IIIa was demonstrated in all isolates with high-level kanamycin resistance, and the *ant*(6)-Ia in five high-level streptomycin resistant isolates. Only *vat*(E) was found in one quinupristin-dalfopristin resistant isolate. **Conclusion:** Although all growth promoters were progressively removed from EU in the course of the last years, in this study it has been detected a high percentage of fecal carriage by *van*A enterococcal strains in Portuguese slaughtered pigs for human consumption. Food animals could be representing a risk for the increasing antibiotic resistance in humans through the food chain.

Keywords vancomycin-resistant-enterococci; antibiotic; resistance

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High-throughput screening for caspofungin synergistic compounds against *Aspergillus fumigatus*

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Aspergillus fumigatus is the main cause of severe invasive aspergillosis (IA), an increasingly frequent opportunistic infection in immunocompromised patients. The mortality from IA ranges around 85% to 50% when early treatment is administrated. In addition, the mortality rate in some patient groups surpasses 90%. IA has become a leading cause of death, mainly among hematology patients and is recognized today as the main fungal infection in cancer patients. To combat this life-threatening infection, only limited numbers of antifungals are available. Amongst them, those most frequently used, amphotericin B and triazole drugs, target the fungal cell membrane, a structure common to all eukaryotic cells.

Caspofungin, the first clinically used echinocandin, is a member of a new class of antifungals that inhibits the enzyme β (1,3)-D-glucan synthase, required for synthesis of the fungal cell wall. The discovery of caspofungin, an antifungal with no target in mammalian cells, represented a significant therapeutic advantage, overcoming a major drawback of standard antifungal therapy. In spite of this, no genuine decrease in the mortality figures of IA was achieved after introduction of caspofungin in clinical trials suggesting that future improvements in the therapeutic results of caspofungin should focus on the discovery of novel compounds displaying synergism with caspofungin.

In this work, we have established the experimental conditions for high throughput screening (HTS) to select novel antifungal compounds from a library containing microbial natural products. Additionally, we have identified in "*in vitro*" tests different samples exhibiting antifungal activity against *A. fumigatus* and samples showing synergism in the presence of a low concentration of caspofungin (0.015 µg/ml).

To optimize and validate the screening method, we assayed 1600 natural extracts from a collection of 80000 extracts. Eight hundred extracts tested were produced by actinomycetes while the remaining 800 extracts analyzed came from fungal cultures. The screening was performed on two strains, *A. fumigatus* ATCC 46645 (wild-type strain) and *A. fumigatus* Δ*akuB*^{KU80} (lacking the non-homologous end joining pathway), generally used in genetic manipulation experiments. Antifungal activity was scored using resazurin, a non-fluorescent blue dye that after reduction is converted to the pink colored highly red fluorescent resorufin. Resazurin is an oxidation-reduction indicator of eukaryotic cell viability.

Results have shown that 2.5% of extracts presented antifungal activity against the wild-type strain *A. fumigatus* ATCC 46645 (inhibition cut-off ≥ 60%). In addition, 1% of extracts analyzed showed synergism with caspofungin, and most of them lacked detectable antifungal activity against *A. fumigatus* when tested alone.

Keywords: *Aspergillus fumigatus*, resazurin, High-throughput Screening (HTS), caspofungin, synergism

Hospital associated methicillin resistant *Staphylococcus aureus* (HA-MRSA) currently prevalent in Central Europe: epidemic but not really multiresistant

Wolfgang Witte; Franziska Layer and Ulrich Nübel

From 1991 to 2000 the procentual incidence of HA-MRSA increased from ~2% to >20% . During this time clonal lineages (ST247, ST228) disappeared, and among newly emerging clonal lineages ST225 and ST22 became particularly prevalent. These strains exhibit a less broad resistance profile: OXA, ERY, CLI, CIP, MFL (*mec*A, *erm*A/*erm*C, mutations *par*C, *gyr*A).

Resistance to other classes of antibiotics is still not frequent:: (ST22/ST225) DAP 0,2/1,7% ;FOS 0/0; FUS 1,9/0% ;GEN 0/4,8%; LNZ 0/0.5%; MUP 0/0; RAM 1,7/4,5%; TGC 0/0; TPL 0/0; VAN 0/0. Of particular interest are isolates exhibiting resistance to daptomycin and in parallel the GISA phenotype

Analysis of single nucleotide polymorphisms (~2 % of the genome, 110 core genome loci, assessed by d dHPLC and subsequent sequencing) gives insights into the evolution of these epidemic clonal lineages which originate from ancestors less abundant in the *S.aureus* population.

MRSA ST225 represent a subpopulation of MRSA CC5 which evolved from MRSA ST5 in the USA ~ 20 years ago and became epidemic in Cenrtral European hospitals after 2002.

MRSA ST22, already known as "EMRSA 15 in the UK, became epidemic in entral Europe after 1996. It presents an epidemic subpopulation of *S.aureus*/MRSA ST22 spreading in hospitals.

CA-MRSA ST22 (PV pos.) represent a separate subpopulation.

The reasons for epidemicity of particular subpopulations are not known so far.

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ICE*Sde*5580 of *Streptococcus dysgalactiae* subsp. *equisimilis*, a novel genetic support for *erm*(T)

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The erythromycin resistance methylase gene *erm*(T) was first described in a poultry isolate of *Lactobacillus reuteri* in 1994, then in group D streptococci, and more recently in *Streptococcus agalactiae* (GBS), *S. pyogenes* (GAS), *Enterococcus faecium,* and *Staphylococcus aureus*. *erm*(T) is located either on plasmids or on the chromosome, and generally confers inducible macrolide-lincosamide-streptogramin B resistance (phenotype $iMLS_B$). In a study of virulence factors, antibiotic resistance and molecular epidemiology of group C and G streptococci (GCS/GGS) from healthy carriers and patients, we first detected *erm*(T) in a *S. dysgalactiae* subsp. *equisimilis* strain from a patient with pneumonia. The strain (Sde5580; *emm* type stG652) was resistant to erythromycin [$iMLS_B$; MIC, >256 µg ml^{-1}] and tetracycline [MIC, 24 µg ml^{-1}; genotype *tet*(M)]. In conjugation experiments with strain Sde5580 as the donor, *erm*(T) was transferable (at high frequency) to *S. pyogenes* 12RF and *S. suis* v36RF recipients; all transconjugants showed an $iMLS_B$ phenotype and were tetracycline-susceptible. SfiI PFGE analysis was consistent with the insertion of a ~70-kb *erm*(T)-carrying mobile element into the genome of the recipients. The mobile genetic element was identified as an integrative conjugative element (ICE*Sde*5580), where the *erm*(T) gene was located on a ~5-kb cargo element. Remarkably, this cargo element, detected by PCR in circular form or integrated in ICE*Sde*5580, was almost identical to the recently described *erm*(T)-carrying broadhost-range replicative plasmid pRW35, which is found in multiple unrelated GAS strains. In the transconjugants, the integration site of ICE*Sde*5580 was the ribosomal protein L7/L12, as recently described in *S. dysgalactiae* subsp. *equisimilis* ICE*Sde*3396, carrying heavy metal resistance genes. ICE*Sde*5580 is a novel genetic support for *erm*(T) and could facilitate its spread among streptococci.

Keywords *erm*(T); *Streptococcus dysgalactiae* subsp. *equisimilis*; Integrative Conjugative Element (ICE)

Identification and characterization of class 1 integrons in *Proteus* spp. from Urinary Tract Infection

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In a survey of 1080 bacteria isolated from the Hospital 12 de Octubre in Madrid (Spain), 795 were identified as *Escherichia coli*, 144 as *Proteus* spp. and 141 as other microorganisms. The incidence of class 1 integrons was determined in the *Proteus* isolated. Integrons were detected in 35 (24.3 %) of 144 *Proteus* spp. by PCR, with primers that targeted integrase genes, resistance to quaternary ammonium compounds, resistance to sulphonamides and cassette regions. PCR and amplicon sequencing of the cassette regions revealed the presence of gene cassettes that confer resistance to a range of aminoglycosides. Of 35 integrons studied, we found seven different class 1 integrons.

The most numerous (17 strains) was *attI-aadA1-attC-qacEΔ1-sulI*, containing three resistance genes: an adenyl transferase gene (*aad*) encoding resistance to aminoglycosides, one encoding resistance to quaternary ammonium compounds (*qacEΔ1*) and the other one resistance to sulphonamides (*sulI*).

Five isolated showed *attI-sulI*, a defective integron with resistance to sulphonamides and lack integrated gene cassettes and resistance to quaternary ammonium compounds.

Four strains showed *attI-sat-attC*, but without *qacEΔ1* and *sulI*, encoding a streptothricin resistance, contained a *sat* gene, which code for streptothricin acetyl transferase.

Another four strains showed *attI-qacEΔ1-sulI*, an empty integron wich keep only the resistance to quaternary ammonium compounds and sulphonamides.

A fifth pattern was *attI-aadB-attC-aadA7-attC-qacEΔ1-sulI*, found in four bacteria. The variable region of this integron contained two adenyl trasferases genes, one encoding resistance to streptomycine and spectinomycine (*aadA7*) and the other one conferring resistance to gentamicine, tobramycine and kanamycine (*aadB*).

The sixth pattern found in four cases was *attI-orfD-attC- aadA7-attC-qacEΔ1-sulI*, containing the typical 5' and 3'-conserved segments, an open reading frame (*orfD*) and also the *aadA7* gene, encoding resistance to streptomycine and spectinomycine.

Only one isolated showed the last integron, *attI-qacH-attC-aadA1-attC-qacEΔ1-sulI*, which variable region contained a resistance to quaternary ammonium compounds, *qacH*, different from *qacEΔ1*, and *aadA1*, conferring resistance to streptomycine and spectinomycine.

This study confirms the occurrence of integrons in bacteria from Urinary Tract Infection exposed to antibiotic selective pressure, integrons which appear with integrated antibiotic resistance gene cassettes. Some of these integrons are described by first time in this paper.

Keywords integrons, *Proteus*, UTI, antibiotic resistance

Identification of Fluoroquinolone and Tetracycline Resistance Mechanisms in *Coxiella burnetii* using N-terminal proteomics

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The aetiological agent of Q fever, *Coxiella burnetii,* is an obligate intracellular bacterium that multiplies within a phagosome-like parasitophorous vacuole. Tetracycline is considered the mainstay of antibiotic therapy while fluoroquinolones have been used as a reliable alternative treatment for the therapy of Q fever patients. Resistance to tetracycline and fluoroquinolones can arise via several mechanisms utilized by pathogens to avoid killing. To date, the studies performed have focused solely on mutations in Quinolone Resistance Determining Regions as the main mechanism of *C. burnetii* to resist inhibition by fluoroquinolones while concerning tetracycline there is no study, up to date, investigating the molecular mechanisms of *C. burnetii* that confer to the bacterium resistance to tetracycline. In this study, in a broader investigation for *C. burnetii* mechanisms that confer resistance to fluoroquinolones (FQ) or tetracycline (TC), *in vitro* developed FQ/TC resistant *C. burnetii* strains were quantitatively compared with susceptible ones on the level of total proteome using the MS-driven COmbined FRActional DIagonal Chromatography proteomics technique. The quantitative comparison of the 381 identified proteins common to both FQ resistant and susceptible strains identified 13 proteins over-expressed in the susceptible strain and 2 proteins over-expressed in the FQ-resistant strain. The following *in silico* analysis of the identified proteins indicated the contribution of a possible efflux pump as an additional *C. burnetii* fluoroquinolone resistance mechanism. The results also suggest the existence of a possible link between fluoroquinolone resistance and decreased cell invasion ability and bring to surface several proteins that may directly or indirectly participate in fluoroquinolone resistance mechanisms of *C. burnetii*. In the case of the TC-resistant and susceptible bacteria 531 proteins common to both samples were identified. The *in silico* analysis of the identified proteins that followed indicated the existence of a ribosomal protection protein in *C. burnetii* (common to other pathogens mechanism of resistance to tetracycline) and brought to surface several proteins that may directly or indirectly participate in tetracycline resistance mechanisms of *C. burnetii*.

Keywords: Q fever, *C. burnetii,* COFRADIC, antibiotic resistance mechanisms, fluoroquinolones, tetracycline

Identification of integrative genetic elements from the human oral metagenome

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The oral microbiota is a complex and diverse community comprising more than 700 different bacterial species, a large proportion of which cannot yet be cultivated. Within this community, mobile genetic elements are widespread, contributing to the evolution and adaptation of these bacteria by encoding functions supplementary to those on the host chromosome. Of these, conjugative transposons, also referred to as integrative and conjugative elements (ICEs), are among the most commonly reported, often carrying genes conferring resistance to antibiotics. These elements all contain site-specific recombinases enabling them to integrate into and excise from host genomes, leading to suggestions that the oral bacteria acquire, and act as a reservoir for, antimicrobial resistance genes. In order to isolate novel elements from the oral metagenome we designed a set of probes complementary to known integrases. These were used to probe a macro-array of a metagenomic library created from saliva DNA samples collected from four European countries. Initial data confirmed the presence of tyrosine-recombinase family integrases, associated with the Tn*916*-like group of conjugative elements, which were linked to genes encoding tetracycline resistance. This method has the potential to detect novel integrative elements and does not require any prior knowledge of accessory genes.

Keywords: conjugative transposon, metagenome, antibiotic resistance

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Implication of efflux pumps in fluoroquinolone resistance of recently isolated *Pseudomonas aeruginosa* and *Staphylococcus aureus* clinical strains

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Staphylococcus aureus and *Pseudomonas aeroginosa* are two species of major clinical relevance because of their increasing resistance to a wide array of antibiotics. Among them, fluoroquinolone resistance (FQ-R) mainly arises through (i) mutations of the antibiotic targets (*i.e*. topoisomerase and gyrase) and (ii) actiive expulsion of fluoroquinolones (FQ) from the bacterial cell through efflux pumps such as NorA and NorB for *S. aureus* and tripartite pumps belonging to the RND family such as MexAB-OprM for *P. aeruginosa.* Overexpression of these pumps could thus partly explain high levels of FQ-R.

To assess the role and clinical relevance of efflux pumps in FQ-R, 115 strains of *P. aeruginosa* and 116 strains of *S. aureus* (57 meticillin-resistant and 59 meticillin-sensitive strains) were retrieved from clinical samples at the Centre Hospitalier Régional Universitaire de Lille between May 2008 and November 2009. E-test was used to determine their MIC for ciprofloxacin (CIP) and levofloxacin (LEV). Using breakpoints recommended by the the E-test manufacturer, 20.9 % and 27 % of *P. aeruginosa* strains were classified as resistant to CIP and LEV, respectively. In addition, 3.5% and 10.4% were classified as having an intermediate sensitivity to CIP and LEV, respectively. As mentioned above, meticillin resistance was determined for *S. aureus* strains, in addition to FQ MICs. All *S. aureus* strains were simultaneously resistant to CIP and LEV and FQ-R very largely correlated with meticillin resistance, as shown in Table 1.

Table 1. Meticillin and fluoroquinolone sensitivities of S. aureus strains.

Meticillin	Number of strains	CIP/LEV		
		S[a]	I	R
S	59	55 (93.2%)[b]	0	4 (6.8%)[b]
R	57	2 (3.5%)[c]	0	55 (96.5%)[c]

a: Breakpoints for CIP were S ≤ 1µg/ml, I = 2µg/ml and R ≥ 4µg/ml

for LEV were S ≤ 2µg/ml, I = 4µg/ml and R ≥ 8 µg/ml

b: Percentage of total meticillin-sensitive strains

c: Percentage of total meticillin-resistant strains

Ciprofloxacin and levofloxacin were selected because of their different hydrophobicities, ciprofloxacin theoretically being a better substrate for efflux pumps. Therefore, *P. aeruginosa* and *S. aureus* strains displaying great discrepancies between MICs for these two FQ would be likely to highly express efflux pumps. A selection of such strains, as well as highly resistant strains to both FQ were further subjected to the determination of ciprofloxacin MIC by the broth dilution technique with or without the addition of an efflux pump inhibitor (phenylalanine-arginine beta-naphtylamide for the RND family or lansoprazole for NorA) to assess the implication of efflux pumps in FQ-R. Preliminary results show that 60% of the tested *S. aureus* FQ-R strains display a decrease in their MIC when ciprofloxacin is combined with lansoprazole (100 µg/ml). However, this decrease is relatively modest (two to four-fold reduction, typically), especially for highly resistant strains. Therefore, although it is relatively frequent in our panel of *S. aureus* clinical strains, efflux is not the primary mechanism supporting FQ-R. Results for *P. aeruginosa* strains are more heterogeneous, as were discrepancies recorded between CIP and LEV sensitivities for these strains. These latter observations warrant further investigations before drawing a conclusion for *P. aeruginosa.*

Keywords *Pseudomonas aeruginosa*, *Staphylococcus aureus*, fluoroquinolone, resistance, efflux pump

In vitro antimicrobial interactions of biocides and antibiotics against multi-resistant strains isolated from organic foods.

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Introduction. Mechanisms of resistance to antimicrobials used to treat infectious disease have been known since antibiotics were introduced into routine clinical usage. Imprudent and often overuse of antimicrobials has, however, compounded the problem by enriching for resistant bacterial populations at the expense of sensitive ones. Then, we are faced with important human pathogens displaying resistance to multiple antimicrobials, and the attendant challenge of treating infections with an ever-dwindling number of effective therapeutic agents. While biocides play an important role in limiting the potential sources of infection and appear yet to be effective, there is concern about the increasing use of biocides in the community and the high resistance development, as well as the potential for cross-resistance to clinically important antibiotics. The aim of this study is to evaluate possible antimicrobial interactions between common used antibiotics and selected biocides against multi-resistant strains isolated from organic foods.

Material and methods. A total of 378 strains were isolated from organic foods using different selective culture media. Three multi-resistant strains were selected (*Enterococcus faecium* and *Enterobacter sp*. isolated from organic whole spelt flour, and *Staphylococcus aureus* isolated from organic carrots) and individual and combined minimal inhibitory concentrations (MICs) of different biocides and antibiotics were determined by the broth micro-dilution method in 96-well microplates as described by the Clinical and Laboratory Standards Institute (CLSI). The interaction of antibiotics with biocides was evaluated by the checkerboard method recommended by the CLSI and expressed as the sum of the fractional inhibitory concentration index (FICI) for each agent. We tested the biocides: 2,2'-methylenebis(3,4,6-trichlorophenol) (CF), didecyldimethylammonium bromide (AB), hexadecylpyridinium chloride (HDPC), cetrimide (CE), and chlorhexidine (CH) in combination with the antibiotics: amoxicillin (AM), erythromycin (EM) and cefuroxime (CX), against those strains which were detected as multi-resistant to some of these antimicrobial agents.

Results and conclusions. In vitro antimicrobial activity of amoxicillin, erythromycin and cefuroxime alone as well as in combination with biocides was tested against those strains which had showed high resistance to these agents. In *Enterococcus faecium*, the MIC (μg/mL) of AB, CE, CF and HDPC alone was 0.1; in combination with amoxicillin this was reduced to 0.00625 (16-fold reduction), 0.0125 (8-fold reduction), 0.0125 (8-fold reduction) and 0.00625 (16-fold reduction), respectively. In combination with erythromycin, it was also reduced to 0.0125 (8-fold reduction), 0.025 (4-fold reduction), 0.00625 (16-fold reduction), and 0.0125 (8-fold reduction). MICs of amoxicillin and erythromycin also showed a 16-fold reduction when combined with all the biocides tested. Thus, synergistic action was found between all antibiotics and biocides studied against this strain (FICI<0.5). Similar results were obtained when all this agents were tested against *Enterobacter sp*. isolated from whole spelt flour.

In *Staphylococcus aureus* isolated from organic carrot, synergistic action was detected between amoxicillin and CE and CF, as well as between cefuroxime and CE and CF, whereas CH with both antibiotics showed neutral results (FICI between 0.5 and 4). In the present study we demonstrate the synergistic interaction between some antibiotics commonly used to treat infectious disease and selected biocides belonging to different chemical categories.

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Keywords: biocides; antibiotics; synergy; multi-resistant strains

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In vitro selection for chitosan-resistant *Staphylococcus aureus* - phenotypic and molecular characterisation of resistance development

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The mounting prevalence of bacterial resistance to antimicrobials is a growing threat that has profoundly impacted the medical community. In this study, we conducted a detailed investigation of the determinants of the *in vitro* development of chitosan-resistance in a well-characterised methicillin-susceptible laboratory strain, *S. aureus* SG511-Berlin (SG511), which reportedly has an insertion mutation in the gene coding for the sensor histidine kinase GraS, which together with its regulatory component GraR forms the two-component system GraRS that mediates *S. aureus* resistance to cationic antimicrobial peptides.

Using an *in vitro* serial passage experiment, we selected for a stable variant of SG511, exhibiting more than 50-fold reduction in its sensitivity towards chitosan (*S. aureus* SG511 chitosan-resistant variant; CRV). We then subjected this isogenic strain set to a series of phenotypic as well as molecular investigations, in order to achieve a better characterisation of resistance determinants. CRV was indistinguishable from the parent strain regarding colony morphology, biochemical activities, cellular ultrastructural morphology, biofilm-forming behavior, phage typing profile and PFGE pattern. However, we identified a cadre of phenotypes that readily distinguished CRV from its parental strain; it displayed (i) modestly increased cell surface hydrophobicity; (ii) altered membrane phospholipid composition, manifested by 6 - 7× higher levels of the positively-charged phospholipid lysyl-phosphatidylglycerol (LPG); (iii) lowering of the overall negative cell surface charge; (iv) lower sensitivity to a number of classical antibiotics as well as prototypic cationic antimicrobial peptides; and (v) higher susceptibility to lysostaphin-mediated lysis. Moreover, binary combinations of chitosan with friulimicin, daptomycin or Pep5 were found to be antagonistic when tested against CRV, but not against the parent strain.

We then compared SG511 and CRV on the transcriptional level in order to identify changes in gene expression patterns that might account for the increased resistance of CRV. This differential gene expression profiling identified a total of 333 ORFs differentially expressed in CRV, as compared to the wild-type strain. It suggested major alterations in the cell envelope structure of CRV, involving an increased biosynthesis of components of the cell envelope and changes in cell surface charge, as well as variations in cellular metabolic pathways.

ORF SA2192 showed a 242× higher expression level in CRV. *In silico* analysis of this ORF (183 bp) revealed that it encodes a hypothetical protein, consisting of 60 aminoacids, with a molecular mass of 6976 and an isoelectric point at 10.57. It is annotated in 13 other staphylococcal species; its GC content lies at 24%, and it does not contain a putative signal peptide sequence. The gene product contains 2 possible transmembrane helices, with an extracellular loop comprising two positively-charged residues. Its RNA-transcript showed no similarity to known small non-coding regulatory RNAs (sRNAs) in staphylococci.

Sequence analysis of *graS* in CRV revealed the absence of the insertion mutation that characterises its parent strain, resulting in a functional sensor histidine kinase GraS, which might explain the lower susceptibility of CRV to cationic antimicrobial substances. However, the repaired *graS* gene cannot by itself account for the observed chitosan-resistance of CRV, since a complemented strain, *S. aureus* SG511 pTX*graS* (with a xylose-inducible gene *graS*), was as sensitive to chitosan as SG511. Reversion of the *graS* mutation might therefore play a rather secondary role in the observed resistance development.

Taken together, both phenotypic and genotypic data confirm the key association of cell envelope structural and functional alterations with *in vitro* development of chitosan resistance in *S. aureus* SG511-Berlin. A reduced overall negative cell surface charge, through the D-alanylation of teichoic acids, as well as the increased production of LPG, together with a repaired *graS* gene are major factors contributing to the decreased susceptibility to the polycationic substance chitosan. Moreover, the cross-resistance to antimicrobial peptides may also indicate a more general resistance mechanism to cationic compounds.

Keywords: chitosan; resistance

Incidence of antimicrobial resistance and virulence factors among food enterococci

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Enterococci are gram-positive bacteria that belong to the normal gastrointestinal microbiota of mammals and other warm-blooded animals, also being found in soil, on plants and in water. By intestinal or environmental contamination they can colonize raw foods, such as milk and meat, and multiply in these materials during fermentation. Many fermented products made from meat and milk, especially fermented meats and cheeses, contain enterococci which are involved in the production of their typical organoleptic characteristics, contribute to their shelf life and improve the hygienic safety, due to the production of antimicrobial substances such as bacteriocins.

Enterococci are clearly at the crossroads of food safety. On one hand, they are present in fermented products and unequivocally contribute to their organoleptic uniqueness but, on the other, they are increasingly responsible for community-acquired and nosocomial infections. Therefore, the presence of enterococci in food poses apprehension regarding their potential pathogenicity and possible transmission via the food chain. In order to address this issue, the aim of this study was to analyze the pathogenicity potential of enterococci isolated from the traditional fermented sausage "chouriço", produced in two processing units at Alentejo, Portugal. After identification at species level and genomic typing of the isolates, the incidence of virulence traits and antibiotic susceptibility/antimicrobial determinants was assessed.

Regarding the screening for virulence factors, one isolate produced cytolysin/hemolysin, four were gelatinase-positive and none produced lipase. Multiplex-PCR for virulence determinants showed the presence of *gelE* in seven enterococci, *esp* in three and the cytolysin activator (*cylA*) in one, while the gene coding for the aggregation substance (*agg*) was absent amongst the isolates under analysis.

Antimicrobial susceptibility testing revealed a multiresistant phenotype in eight of the isolates, six of which from factory A. The antimicrobial resistance gene *ere(B)* was detected in nine isolates, whereas each of the genes *tet(M)*, *aac(6')-Ie-aph (2")* and *vanA* was detected in eight isolates, from both sampling sites.

Although virulence determinants were mainly absent among *E. faecalis* "chouriço" isolates, some of them present a multiresistant profile and harbor resistance genes. So, to further assess for the food safety of the Portuguese dry sausages, screening of virulence and antimicrobial resistance traits must be performed in a larger number of meat products and processing units.

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Linezolid and multiresistant Tuberculosis, a new option in children

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Linezolid is a new antimicrobial from a new family, oxizolidinones that are very useful in gram positive infections with little experience in children. It has been published that is also useful for Mycobacterium Tuberculosis infections but in this case there is no report of this treatment in small children. Children with resistant tuberculosis are difficult to treat because there are few antimicrobial approved for this age.

We present what we think is one of the first cases of a girl 16 months old with pulmonary multiresistant tuberculosis that was treated one year with Pirazinamid, Clarytromicin and Linezolid with excellent results and no secondary effects.

Linezolid is very easy to give because is an oral syrup, BID, and is well tolerated.

Its worst effect is haematological suppression and that must be controlled. It is also very expensive.

Because of that, we think that this treatment must be considered when no other alternatives exist.

Keywords: Tuberculosis, Therapeutics, Child, Oxazolidinones

Linezolid Resistance in Two Coagulase Negative Staphyloccus Species, Isolated at the Same Time was Associated with the Same G2603T Mutation

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Background: To describe the resistance molecular mechanisms of different linezolid-resistant coagulase-negative staphylococci (CoNS) species isolated in a 6 month period in high risk units.

Methods: Linezolid resistant strains (13 S. epidermidis and 2 S. hominis) were isolated from 14 patients admitted in high risk units from October 2009 to March 2010. A patient carried the two different species in the same sample. Identification and susceptibility was determined using Vitek2®. Activity of linezolid and daptomycin were confirmed by E-test®. Strains were genotyped by PFGE. Resistance mechanisms to linezolid were determined by amplification and sequencing of the domain V of 23S rRNA gene and presence of the cfr gene by PCR.

Results: Ten isolates (71.4%) were recovered from blood cultures. All isolates were resistant to oxacillin, clindamycin, gentamicin, rifampin, cotrimoxazole, showed intermediate susceptibility to levofloxacin, and susceptibility to erythromycin, vancomycin, tigecycline and daptomycin. MIC to linezolid was >256 mg/L in all strains and CMI90 for daptomycin was 0.25 mg/L. The S. epidermidis isolates showed the same PFGE pattern and the two S. hominis were also genetically indistinguishable. Surprisingly, sequence of amplified domain V of 23S rRNA of these two different clones of CoNS showed the same mutation G2603T, recently described. There was not amplification for cfr gene.

Conclusions: 1.- The same mutation in domain V of 23S rRNA gene was responsible for linezolid resistance in two clones of different species of CoNS isolated during the same period. 2.- Not MLSB phenotype was detected and clindamycin resistance was likely due to a conformational change in drug target site as a result of the new mutation. 3.- Person to person spread of a single strain was likely the origin of the infection/colonization cases.

Keywords linezolid; resistance

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Lysin modification of the membrane lipid phosphatidylglycerol of *Staphylococcus aureus* confers resistance to antimicrobial peptides and impairs peptide insertion and pore-formation in model membranes

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Modification of membrane phosphatidylglycerol (PG) of *Staphylococcus aureus* by addition of a L-lysine residue changes the net charge of this lipid from -1 to +1 and confers resistance to cationic antimicrobial peptides (AMPs) such as the human defensins [1]. AMPs are part of the innate immunity and provide the first line of defence against pathogenic microorganisms. Due to their unique mode of action, they have gained high interest as new drugs to overcome bacterial resistance to classical antibiotics. The primary target of AMPs is the cell membrane of bacteria. It is widely accepted that the peptides cover the bacterial membrane like a carpet and induce the formation of transient or stable pores. However, their precise mode of action and the significance of the natural lipid composition is still a matter of debate.

We investigated the ability of three different AMPs and derivatives thereof to inhibit the growth of a clinical isolate of *S. aureus,* wild type strain SA113, and, since PG lysination is done by the membrane protein MprF, mutants lacking the MprF gene. Moreover, we visualized ultrastructural changes by electron microscopy and correlated the biological activity with peptide insertion, analyzed by Förster resonance energy transfer (FRET) spectroscopy, and pore formation in planar lipid bilayers composed of PG, lysyl-PG, and phosphatidylethanolamine. These lipids were chosen to mimic the lipid compositions of the cytoplasmic membranes of various *S. aureus* strains. The studied peptides were: i) NK-2 [2], an α–helical fragment of mammalian NK-lysin, ii) arenicin-1 [3], a lugworm cyclic β–sheet peptide, and iii) bee venom melittin.

For all peptides, bacterial growth inhibition and biophysical data obtained from the model systems were consistent, suggesting that peptide-membrane interaction is crucial for killing *S. aureus*. The ability of NK-2 to inhibit the growth and to kill *S. aureus* was strongly dependent on the amount of lysyl-PG in the membrane. Furthermore, an impaired antibacterial activity was paralleled by a reduced capacity to insert into liposome membranes and to form pores in planar lipid bilayers, demonstrating that PG lysination strongly affect membrane interaction of the cationic peptide NK-2 and effectively protects bacteria against the action of this peptide. In contrast, the biological activity of melittin as well as its interaction with model membranes was only marginally affected by lysination of PG, indicating that interaction of NK-2 is primarily driven by electrostatic forces and melittin interaction is not. Arenicin-1 was also affected by lysination, however, was still considerably active against *S. aureus* strains which expose lysyl-PG and is thus the most promising lead structure for drug development.

Keywords antimicrobial peptides; arenicin, lysyl-phosphatidylglycerol; melittin; model membranes; NK-lysin; planar lipid bilayer; Staphylococci

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Mechanism(s) underlying various response of multiresistant *Staphylococcus aureus* to photodynamic inactivation

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Background: Photodynamic inactivation (PDI) is based on a concept that a non toxic chemical, named a photosensitizer upon excitation with light of appropriate wavelength is activated. As a consequence singlet oxygen and other reactive oxygen species are produced, which are responsible for the cytotoxic effect towards bacterial cells. Photodynamic inactivation can eliminate bacteria from the environment (eg. human wound), therefore it is proposed as an alternative option of killing of multiresistant microorganisms, like *Staphylococcus aureus*. It is of great clinical importance and an advantage of PDI that *S. aureus* isolates, including multiresistant *Staphylococcus aureus* (MRSA), can be effectively killed with the use of PDI. Previous reports of our group emphasized that *S. aureus* response to PDI is a strain-dependent phenomenon, which from clinical point of view warrants attention (Grinholc *et.al.* 2008). In our attempts to determine the molecular marker of strain-dependent response to PDI, we found that biofilm producing strains were killed less efficient in comparison to non biofilm-producing strains, whereas efflux pumps, eg. NorA had no influence on the efficacy of photokilling. In the presented work we focused on the role of superoxide dismutases (Sod) in the response of *S. aureus* to PDI.

Methods: In our current study we checked the survival rate of *S. aureus* clinical isolates as well as reference strains deprived of Sod activities in response to PDI. Protoporphyrin IX, a heme precursor, was used as a photosensitizer. Protoporphyrin IX was excited with red light (wavelength 623±18nm) with illumination dose of 12 Jcm^{-2} (BioStimul SS01 lamp, Biotherapy, Czech Republic). Total Sod activity was measured with NBT (nitroblue tetrazolium) reduction assay. Using quantitative real-time PCR technique we also measured a transcript level of both *S. aureus* superoxide dismutase genes, namely *sodA* and *sodM*. Correlation between Sod activity, Sod transcript level and *S. aureus* response to PDI was checked statistically.

Results: The survival rate of *S. aureus* Sod mutants differed significantly according to *Sod* gene status. We found that among 8 clinical isolates tested, 4 were recognized as PDI-sensitive and 4 were classified as PDI-resistant. The mean value of Sod activity in PDI-sensitive strains was about 4 times higher after PDI treatment in comparison to PDI-resistant strains. This result was statistically relevant and consistent with the increase in *sodA* and *sodM* genes transcript levels.

Conclusion: Sod activity increases after photodynamic inactivation treatment but only in PDI-susceptible strains

Keywords photodynamic inactivation; *Staphylococcus aureus*; superoxide dismutase

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Methicillin resistance in *Staphylococcus aureus* isolated from health care personnel

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Staphylococcus aureus is known as a pathogen responsible for skin infections and invasive diseases such as meningitides or pulmonary infection. Resistance to antibiotics is in fact a problem and methicillin-resistant *Staphylococcus aureus* (MRSA) have been isolated all over the world, including in health care staff. In the present study, 336 samples from the nasal cavity and from the hands of health professionals from a hospital located in the North of Portugal were collected for the screening of MRSA. Two hospital services were investigated: surgery and medicine.

One hundred and sixty eight volunteers including doctors, nurses and auxiliaries were analyzed. Swabs were spread onto Baird-Parker Egg Yolk Tellurite Agar and further incubated at 37 ºC during 48h. Characteristic colonies were then selected and confirmed as *S. aureus* (43.5%, n=73, from the nasal cavity and 15%, n=26, from the hands). The susceptibility to oxacillin was determined according to CLSI (2009) guidelines. Resistance to other β-lactams, namely penicillin and ampicillin, was investigated. The presence of the methicillin resistance gene (*mecA*) was also evaluated.

According to our results, *S. aureus* isolated from the nasal cavity and hands of professionals, 52% (38/73) for nasal cavity and 53.8% (14/26) for hands were considered MRSA. Among the volunteers 22.6% (38/168) and 8.3% (14/168) carried MRSA in the nasal cavity and in hands, respectively. Doctors were the professionals showing the lowest levels of contamination by MRSA.

Keywords MRSA; health-care personnel

Methicillin resistant staphylococci (MRS) in canine pioderma: control proposals.

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A study to determine the antimicrobial susceptibility of staphylococci isolated from dogs and the risk factors associated to the resistance was performed. A total of 74 dogs submitted to the Clinical Veterinary Hospital of Cordoba, from October to December 2009 were analyzed. Three groups were established. Group 1 included 23 healthy dogs and group 2 included 24 dogs with a first-time pioderma, both without antibiotic therapy at the last year. A third group included 27 dogs presenting a recurrent pyoderma and they had received long-time antibiotic treatments. The following data were collected from the animals: breed, sex, age, habitat, cohabitation with other animals and other dogs. The resistance to Oxacillin was determined on a selective media Oxacillin Resistance Screening Agar Base (ORSAB, Oxoid S.A., Spain) and later confirmed by a latex agglutination assay, detecting PBP2a (Oxoid, S.A., Spain). The antimicrobial susceptibility, using the disk diffusion method against 8 different groups of antimicrobials was studied. Likewise, the clindamycin disk induction test (D-test), was performed to determine if the microorganism is truly susceptible to clindamycin, or whether there is a risk of inducible macrolide-lincosamide-streptogramin$_B$ (MLSB) resistance. For the study of risk factors associated with antimicrobial resistance, multiresistance and methicillin-resistance, the percentage of resistant isolates in each group and their confidence intervals (95% CIs) were determined and compared using the Fisher´s exact test and Odds Ratio (ORs), with a statistical significance of 5%.

A total of 126 isolates were obtained, identified by biochemical test as SIG group (81%), *S. aureus* (8%) and CoNS (11%). We detected an 8.7% of methicillin-resistant staphylococci, and the probability to isolate methicillin-resistant staphylococci was 7 times higher in dogs with recurrent pyoderma (95% CI [1.4, 33.9]). Among the methicillin-resistance isolates, resistance to fluoroquinolones, macrolides, aminoglucosides and tetracyclines was detected. Multiresistant isolates were obtained more commonly from urban than rural dogs (OR 3.2, 95% CI [1.03, 10.1]) and all the methicillin-resistant staphylococci were obtained from urban dogs. In this paper, the increase of resistance of staphylococci to drugs widely used as first-line of treatment in pyoderma cases is showed, for that, the study of susceptibility of the isolates in cases of recurrent pyoderma is always advisable. Sixteen isolates showed an unusual pattern in the Kirby-Bauer test (ERY-resistant but CLI-susceptible). Of the 16 isolates with an unusual pattern for erythromycin-clindamycin, two showed inducible resistance. These results support the routine use of the D-Test to value the efficacy of the Clindamicin for the treatment of pyoderma, especially in cases associated with methicillin-resistant isolates.

Keywords Staphylococci, pyoderma, dog, antimicrobial susceptibility, methicillin-resistance

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Methicillin-resistant *Staphylococcus epidermidis*: SCCmec and MLST of prevalent and sporadic genotypes isolated from hospitals in Rio de Janeiro, Brazil

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Staphylococcus epidermidis is the most frequent nosocomial pathogen associated with indwelling medical device-related infections and methicillin resistance rates higher than 70% have been reported in hospitals worldwide. Prevalent clones of methicillin-resistant *S. epidermidis* (MRSE) have been described, but a complete molecular characterization of isolates has rarely been performed. The objective of this study was to characterize by multilocus sequence typing (MLST) and to detect the staphylococcal cassette chromosome *mec* (SCC*mec*) in 35 MRSE isolated from seven hospitals in Rio de Janeiro, between 1994 and 1999. The strains were previously classified by our group, using the pulsed-field gel electrophoresis (PFGE), into 22 isolates of two prevalent genotypes (A and B) and 13 isolates of 11 sporadic genotypes (C to M). Eleven isolates of genotype A were SCC*mec* III (85%), and seven isolates of genotype B, SCC*mec* IV (87%). The isolates of sporadic genotypes were 64% nontypeable. All isolates from prevalent genotypes were included into the major clonal complex (CC) of *S. epidermidis*, CC5. The genotype A isolates were classified into the major sequence typing (ST) of *S. epidermidis*, ST2. While the genotype B isolates were classified into ST 23, except for one that was included in ST 231. In the present study we described one new allele (26 of *mutS* gene) and two new STs, 231 and 263. The 13 isolates from sporadic genotypes were included into three CCs, but the CC5 was associated with the majority of them (77%). These isolates were included into 8 STs (2, 22, 23, 53, 59, 81, 237 e 263). We can conclude that the MRSE isolates of prevalent genotypes A and B, in Rio de Janeiro hospitals are respectively ST2 associated with SCC*mec* III and the ST23 associated with SCC*mec* IV.

Keywords *Staphylococcus epidermidis*; methicillin; PFGE; SCC*mec*; MLST

Mismatch repair deficiency of multiresistant, extended-spectrum β-lactamase producing Esherichia coli from community-aquired urinary tract infections

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The emergence of community acquired urinary tract infections (CAUTI) caused by multiresistant ESBL producing *Escherichia coli* is increasing. Recently, new concepts have been developed to understand the emergence and spread of antimicrobial resistance. These include, among others, the discovery of the hypermutators in natural populations of bacterial pathogens. Hypermutable bacteria have an increased spontaneous mutation rate due to defects in genes involved in DNA repair. Most spontaneous hypermutators appear to be defective in postreplicative mismatch repair (MMR).

The aim of this study was to determine the relationship between multiresistance and hypermutator phenotype of the extended-spectrum β-lactamases (ESBL) producing *Escherichia coli* isolates from children with community aquired urinary tract infection (CAUTI) and to compare them with the non-ESBL producing resistant isolates as well as with antibiotic susceptible ones.

The strains were randomly chosen from a large clinical collection of *E. coli* isolates from children not-hospitalised nor treated with antibiotics previously. According to well defined inclusion and exclusion criteria, based on resistance patterns, samples were asigned to 3 groups: 1) multiresistant ESBL producing, 2) resistant but not ESBL producing and 3) susceptible to all antibiotics tested. Isolates were considered multiresistant if they were resistant to more than two other antimicrobials (gentamicin, amikacin, ciprofloxacin or cotrimoxazole). Non-ESBL were resistant to either ciprofloxacin or cotrimoxazole. ESBL producing strains studied were not epidemiologically related.

In the present study we have examined the frequency of mutation to rifampicin. Laboratory strains AB1157 and GM1760 (as AB1157 but *mutS260*::Tn*5*) were used for wild type and mutator strain respectively. Isolates were considered mutators if they exhibited frequencies of mutations conferring resistance to rifampicin (100 μg/ml) that were 10-fold higher than the median value of mutagenesis (5.04×10^{-9}) observed for all studied strains (n = 150). Mutation frequencies ranged between $1{,}01 \times 10^{-9}$for non-mutator strains to $7{,}41 \times 10^{-4}$ .for hypermutators. Among tested isolates 31,76% have incresed mutation frequency and 4,05% were hypermutators. Multiresistant ESBL-producing *E.coli* CAUTI isolates had a higher proportion of hypermutators compared to non-ESBL resistant and antibiotic-susceptible isolates. General mutator phenotype was confirmed by measuring the frequencies of mutations that confer resistance to five additional antibiotics. Wildtype *mutS* and *mutL* genes were used to supresss hypermutator phenotype. Greater than 97% suppression of the mutator phenotype as assessed by spontaneous mutagenesis to rifampicin resistance was considered as significant positive complementation. Analysis showed that five putative mutators belonging to multiresistant ESBL-producing group contained a defective MMR allele, they were confirmed to be *mutS* deficient.

This study demonstrates hypermutability of epidemiologically non-related multiresistant ESBL-producing *E. coli* strains isolated from CAUTI and indicates that hypermutator phenotypes may influence the multidrug resistance development in the absence of treatment-related antibiotic selective pressure.

Keywords multiresistant *Escherichia coli*; ESBL-producing; CAUTI, hypermutators

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Molecular analysis of antibiotic resistant *Mycobacterium tuberculosis* strains in Central West Brazil

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Introduction: Multi drug resistant (MDR) strains of *Mycobacterium tuberculosis* threaten the global control of tuberculosis (TB), since treatment of those strains are complex and expensive with higher rates of failures and side effects. Diagnosis of resistance by molecular techniques is faster than conventional microbiological tests, and can provide promptly appropriate and aggressive therapy. **Objectives**: To determine the frequencies of mutations observed in the rpoB and katG genes obtained by sequencing the genomic regions known to have high frequency of mutations, and compare with the phenotypic resistance profiles of *M. tuberculosis* strains from patients of Goiás State, Brazil. **Methods:** The clinical charts were reviewed in order to collect the patient's clinical data. Samples were processed by culture in Lowenstein-Jensen (L-J). *M. tuberculosis* isolates were tested for sensitivity using the method of Canetti et al (1969). Extraction of chromosomal DNA was performed by the technique described by van Soolingen et al (1994). The rpoB and katG genes were amplified with the primers described by Siddiqi et al (2002) and Martilla et al (1996), respectively. The purified products were sequenced with Big Dye Terminator kit (Applied Biosystems) and detection of sequences performed in an ABI3130 apparatus (Applied Biosystems). Sequencing reactions with the forward and reverse primers were analyzed using the DNASTAR software, version 5.1 (licensed to Fields Center for Disease Control) for the assembly of contigs. Additionally mutations in the mab-inhA operon were analyzed by RT-PCR. **Results**: During the period of September 2005 to December 2007, 410 *M. tuberculosis* positive cultures were identified from a total of 748 cultures performed at the reference laboratory (LACEN-GO) of Goias. Surprisingly, only 30% (124 isolates) of the cultures were tested for antimicrobial susceptibility because, according to the Brazilian tuberculosis control program, cultures are tested only upon the physician´s request. According to Canetti`s susceptibility test, 16 patients had *M. tuberculosis* cultures resistant to either H or R. Among those, ten patients had MDR-TB. Twenty four isolates obtained from 16 patients were analyzed for mutations. Mutations in codon 315 of the katG gene and in the mab-inhA operon accounted for 45% and 24%, respectively of the H resistance, while mutation in the rpoB gene accounted for 91% of the R resistance. Two patients that had MDR isolates in the first sample, presented monoresistant strains in the following samples, indicating the possibility of infection with mixed *M. tuberculosis* strains. **Conclusions**: The high level of resistance observed, indicates that a more thorough screening of resistance for *M. tuberculosis* isolates should be made. The molecular approaches should be implemented in Goias in order to shorten the time needed for resistance identification.

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Molecular analysis of reduced telithromycin susceptibility of *Streptococcus pneumoniae* collected clinically between 2005 and 2009 in Japan

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Streptococcus pneumoiniae is an important pathogen that has been identified as a primary cause of community-acquired pnemonia (CAP). Macrolides are commonly used for treat CAP, however, during the last decade, the rates of macrolide resistance among the species have been increasing worldwide. Telithromycin (TEL) is a ketlide antibiotic which is potent against *S. pneumoiniae* with high-level resistance to macrolides. In the present study, a total of 526 *S. pneumoiniae* isolates collected between 2005 and 2009 in Japan were examined for susceptibility to TEL and 14-, 15- and 16-membered ring macrolides. MICs ranged for all macrolides tested from <0.125 μg mL^{-1} to >128 μg mL^{-1}. The MIC_{50}s of erythromycin, azithromycin, spiramycin and rokitamycin were 32 μg mL^{-1}, >128 μg mL^{-1}, >128 μg mL^{-1} and 0.5 μg mL^{-1}, respectively. Although none of the isolates were assigned as TEL-resistant (breakpoint, >4 μg mL^{-1}), 82 isolates had low-level TEL susceptibility, with MICs of 0.25-1 μg mL^{-1} (MIC_{50} 0.06 μg mL^{-1}, MIC_{90} 0.25 μg mL^{-1}), suggesting that pnuemococci with reduced TEL susceptibility have appeared clinically in Japan. The macrolide-resistant determinants in all isolates were analyzed. All isolates were negative for *ermA*, *ermC*, *mphA*, *mphB*, *ereA* and *ereB*. The rates of *ermB*-positive, *mefA/E*-positive and double *ermB* and *mefA/E*-positive isolates were 55%, 33% and 8%, respectively. Interestingly, all the isolates exhibiting reduced TEL susceptibility (MIC 1 μg mL^{-1}) harbored *mefE-mel*, which encodes the macrolide efflux genetic assembly and several isolates also harbored *ermB*, which encode rRNA methylase. Allele replacement mutagenesis of the corresponding genes revealed that reduced TEL susceptibility in *S.pneumoniae* may be caused by acquisition of the *mefE-mel* element only and additionally conferred by the *ermB* determinant. It was previously demonstrated that high-level TEL-resistance was easily generated from macrolide-resistant *S. pneumoniae* harboring *ermB* and *mefA/E*. It is therefore worth mentioning that the reduced TEL susceptibility clones demonstrated in the present study may have the potential to generate TEL-resistant pneumococci and spread further.

Keywords *S. pnemoniae*; Telithromycin Resistance; *ermB*; *mefE*

Molecular characterization of extended-spectrum beta-lactamase producing *Escherichia coli* isolates from human clinical samples

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Nowadays, we are witnessing an emergence of multiple antibiotic resistances in *Escherichia coli* isolates. With this study we intended to determine the type of extended-spectrum beta-lactamases (ESBLs) of isolates from human clinical samples, as well as to characterize other resistance associated genes and phylogenetic groups of each isolate. The susceptibility to 16 different antibiotics of 34 *E. coli* isolates was carried out by disk diffusion agar. The confirmation of ESLB phenotypes was performed by double-disk test and genes encoding TEM, OXA, SHV and CTX-M type beta-lactamases, as well as other resistance mechanisms and phylogenetic groups, were studied by PCR. The following ESBLs were detected: TEM (31 isolates), OXA (12 isolates), CTX-M-3G (29 isolates) and CTX-M-9 (4 isolates). The *aac* (3)-II was identified in 22 of 24 gentamicin resistant isolates; *aad* A gene was found in 17 of the 22 spreptomycin resistant and intermediate-resistance isolates; *tet*(A) or *tet*(B) were detectd in 27 from 28 tetraciclin resistant isolates; *sul* 1, *sul* 2 and *sul* 3 were detected in 8, 6 and 8 of the 19 sulfamethoxazole/trimethoprim resistant isolates, respectively. 30 isolates belong to the B2 phylogenetic group, 3 to B1 and 1 to D. This study demonstrated that commensal microorganisms such as *E. coli* show multiple resistances to several antibiotics of common use, in human patients.

Keywords: antibiotic; clinical; *Escherichia coli*; extended spectrum beta-lactamase; resistance

Monitoring of antimicrobial resistance in zoonotic bacteria in Germany

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Resistance to antimicrobials in zoonotic bacteria is of special concern since these pathogens may compromise the effective treatment of infections in humans. To follow the occurrence of antimicrobial resistance in *Salmonella* isolates from different sources are analysed regularly for risk assessment purposes.

Overall, 33625 *Salmonella* isolates originating from feed- and foodstuffs, animals, and environmental sources collected between 2000 and 2008 were tested by using the microdilution method. The quantitative data were interpreted using harmonised epidemiological cut-off values.

Resistance to antimicrobials was commonly found among *Salmonella* isolates from all sources in Germany. Resistance to commonly used antimicrobials as sulphonamides, tetracycline, ampicillin and some aminoglycosides were frequently observed. Resistance to ciprofloxacin and nalidixic acid were found in 7.2 % and 7.7 % of isolates, respectively. Resistance to 3rd generation cephalosporins was tested with three substances and observed in 0.4 % to 1.1 % of the isolates. The proportion of resistant isolates varied among the sources and *Salmonella* serovars considerably.

The observed ciprofloxacin resistance levels in *Salmonella* isolates, which are markedly high in certain populations, are of concern since fluoroquinolones are critically important antimicrobials in human medicine. Resistance to third generation cephalosporins, another critically important antimicrobial group, observed in some of the *Salmonella* isolates tested needs close monitoring and assessment.

Keywords *Salmonella* spp.; antimicrobial resistance; epidemiological cut-off values; zoonotic agents; risk assessment

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MRSA in wounds of patients attended in primary care

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Staphylococcus aureus is an important bacteria involved in community-acquired infections and hospitals, currently standing out as a major clinical and epidemiological problems in nosocomial infections. In recent years, emerged S. aureus resistant to methicillin community associated (CA-MRSA), that most patients had no hospitalizations within a year and no surgical procedure.

Given the importance of a S. aureus as the predominant microorganisms isolated from skin infections and soft tissue infections and the increasing spread of S. aureus resistant to methicillin (MRSA), which complicates treatment, increases costs and causes great concern, this study aims to identify the presence of MRSA in wounds of patients attended primary care units in the city of Botucatu through the characterization of the staphylococcal cassette chromosome mec (SCCmec) and analysis of clinical and socio-demographic characteristics of these patients.

This study included 80 isolates from 70 patients treated between March to August 2010. The samples were submitted for identification and detection of oxacillin resistance by the diffusion method with oxacillin and cefoxitin, penicillin, levofloxacin, clindamycin, erythromycin, gentamicin, sulfametazol / trimethoprim, tigecycline, fusidic acid, quinupristin / Dalfopristin, Linezolid and vancomycin and the characterization of SCCmec by multiplex PCR in samples of S. aureus with mecA. Samples of S. aureus were also submitted by D test for detection of clindamycin resistance.

Of the 80 samples studied, 47 (58.7%) were identified as Staphylococcus spp, these 34 (42.5%) were identified as S. aureus and 13 (16.3%) were identified as coagulase-negative staphylococci (CNS). The mecA gene was found in two (5.8%) isolates of S. aureus and 9 (69%) isolates of CNS. The characterization of SCCmec revealed one sample type IV and one type II for S. aureus and to CNS, six (66.7%) samples were characterized as type III, two (22.2%) samples as type IV, and a sample was not typed. The drug sensitivity test disk showed 9 (19.2%) strains of staphylococci resistant to oxacillin, 40 (85.2%) resistant to penicillin, 17 (36.2%) resistant to erythromycin, 10 (21.3 %) were resistant to gentamicin, 9 (19.2%) resistant to clindamycin, 8 (17%) strains resistant to cefoxitin, 6 (12.8%) strains resistant to levofloxacin, 3 (6.4%) strains resistant to sulfametazol / trimethoprim and 2 (4.2%) strains resistant to tigecycline. Only two strains were sensitive to all drugs, 22 (46.8%) were resistant to only one drug, 10 (21.3%) strains were resistant to two drugs, 4 (8.5%) were resistant to three drugs and 9 (19.2%) strains were resistant to more than four drugs. All strains of CNS resistant to more than four drugs were positive to gene mec A, and two samples carrying the gene were sensitive to the oxacillin disc diffusion method, revealing heteroresistance.

The results from this study shows a high number of CNS resistant to oxacillin, with a predominance of staphylococcal cassette chromosome type III, more common in hospital strains and inserted in the community explained by being chronic wounds and patients with a history of multiple hospitalizations over of life. There were great similarities between strains of Staphylococcus found and the sensitivity of the drugs tested, when comparing samples found in the same basic health unit, which may indicate the presence of a possible clone of Staphylococcus in several patients. The results also call attention to the high rate of multidrug resistance found in strains isolated these patients with predominance of SCCmec type III which complicates the treatment these infections.

Keywords: Staphylococcus, wounds, resistance, MRSA.

Multi-Drug Resistant Acinetobacter baumaunnii: Identification, Isolation and Elimination in an Acute Care Setting

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Background

Multi-drug resistant Acinetobacter baumannii is a health care-associated pathogen that can live for months in both a wet and dry environment. It can live for months on all types of environmental surfaces, i.e. bedrails, faucet handles, door handles, sinks, toilets, making it difficult to eliminate. The high prevalence of this organism in the hospital environment results in colonization of the skin and respiratory tract in the patient population, which can lead to development of infection. MDR Acinetobacter is difficult to treat due to resistance of carbapenems and other standard antimicrobial agents limiting available treatment options. Mortality due to MDR Acinetobacter baumaunnii can run as high as 80% in patients with health-care associated pneumonia and bacteremia.

Methods

A case-only study was conducted over a 12-month period. Interventions used to reduce the incidence of healthcare associated Acinetobacter baumannii included 10% hypochlorite disinfection, hand hygiene, special contact isolation for suspected and confirmed cases, educational tool for clinicians, patient and visitors, daily isolation rounds, automated report functions, and standardized nursing unit isolation practices. Pulse-field gel electrophoresis was performed on all isolates to determine if there was a common genotype among the patient population. Sputum and endotracheal aspirate samples were evaluated by Gram stain smear for absence or presence (less than 10 per low power field) of epithelial cells to ensure that only uncontaminated samples were processed for culture. Using a swab or pipette, a portion of the sample was inoculated to a Trypticase Soy Agar with 5% Sheep Blood (blood agar), chocolate agar and MacConkey agar plates (Becton Dickinson and Co, Franklin Lakes, NJ) and incubated in an incubator at colonies suggestive of Acinetobacter. Acinetobacter spp. isolates were subcultured to Trypticase Soy Agar (TSA) slants (Becton Dickinson and Co, Fair Lakes, NJ). After overnight incubation, they were transported for genotyping.

Results (including some data)

There were a total of eighty-five (85) isolates collected during the 12-month period. 52 (61%) were healthcare-associated and 33 (39%) were community acquired. The most prevalent genotypes identified were Type J and Type O at twenty-six percent (26%) and twenty-two percent (22%) respectively. In the first month of implementation of a new protocol to collect respiratory specimens on admission from other acute care facilities, there was an 87.5 % reduction in healthcare-associated isolates.

Conclusions

A combination of an admission screening protocol of patients transferred from other acute care facilities, implementation of a 10% hypochlorite disinfection protocol and isolation of those patients at time of admission until negative culture results can prevent transmission of healthcare-associated and community acquired MDR Acinetobacter in a healthcare entity.

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Multidrug resistant *Pseudomonas species* isolated from skin lesions of patients from a tribal area in India

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Introduction: *Pseudomonas* is a Gram-negative rod bacterium that belongs to the family Pseudomonadaceae. More than half of all clinical isolates of *Pseudomonas* produce phyocyanin, a blue-green pigment. In most cases of infection, the integrity of a physical barrier to infection (eg, skin, mucous membrane) is lost or an underlying immune deficiency is present. Adding to its pathogenicity, this bacterium has minimal nutritional requirements and can tolerate a wide variety of physical conditions. It is a clinically significant and opportunistic pathogen, often causing nosocomial infections. In addition to causing serious and often life-threatening diseases, these organisms exhibit innate resistance to many antibiotics and can develop new resistance after exposure to antimicrobial agents. Some *Pseudomonas* species that previously were considered the causative agents of old diseases, now are being reexamined for their potential use as biological warfare agents.

Objective: We have conducted this study to find out the antibiotic susceptibility pattern of *Pseudomonas*. This will help our clinicians in prescribing appropriate treatment against skin infections caused by *Pseudomonas*.

Period of Study: The samples were collected from March to June 2010 from tribal areas of India.

Method: Clinical specimens were skin lesions of the patients. *Pseudomonas species* were isolates by enrichment of tissue fluid of the patients and further identified by biochemical tests and standard PCR method. A total of 28 antibiotics were used against *Pseudomonas species*. Antimicrobial susceptibility test was performed using Kirby-Bauer disc diffusion method.

Results: A total 5 *Pseudomonas species* were isolated from tissue fluid samples of 22 patients having skin lesions. Majority of the isolates were resistant to amoxicillin, amphotericin, ampicillin, cefixime, cefoxitin, chloramphenicol, clarithromycin, clindamycin, colistin, co-trimoxazole, doxycyclin hydrochloride, erythromycin, kanamycin, linezolid, nalidixic acid, oxacillin, penicillin- G, rifampicin, roxithromycin, streptomycin, tetracyclin and vancomycin and moderately sensitive to meropenem and tobramycin. However, the isolates were sensitive to ciprofloxacin, gentamycin, imipenem and norfloxacin.

Conclusion: Emergence of resistance in *Pseudomonas species* for most of the antibiotics is a challenge for our clinicians. Only few antibiotics like Ciprofloxacin, Gentamycin, Imipenem and Norfloxacin prove to be effective for the treatment hence quit difficult to cope up with the emerging resistant strains for the overcome of *Pseudomonas* outbreaks in future days. A continuous monitoring of the *Pseudomonas* isolates for the drug resistance is important for the clinical point of view.

Keywords: Antimicrobial agent, *Pseudomonas.*

Observations on the antimicrobial susceptibility of *Staphylococcus pseudintermedius* following the introduction of cefovecin for clinical use in Europe

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In vitro susceptibility tests were conducted on field isolates of *Staphylococcus pseudintermedius* from canine clinical cases isolated from European countries during 2009-2010 for sensitivity to cefovecin and a panel of other commonly used antimicrobials by broth microdilution and oxacillin disc diffusion tests. Isolates suspected of multi-drug resistance were screened for the presence of the *mecA* gene. Eight isolates with zone diameters of less than 16 mm in the oxacillin disc diffusion test were deemed resistant to oxacillin and, following genotyping, seven were confirmed *mecA* positive. The MIC_{90} and MIC_{50} values for cefovecin were both 0.25 µg/mL and those isolates selected for genotyping had MIC values for cefovecin of ≥1 µg/mL. The results for cefovecin were compared with data from a similar survey conducted prior to launch in 1999-2003. The data indicate that to date for canine clinical isolates of *S. pseudintermedius*, there has been no apparent shift in susceptibility to cefovecin amongst isolates from first opinion cases across Europe but continued monitoring is recommended.

Keywords cefovecin; canine; *Staphylococcus pseudintermedius* susceptibility; methicillin resistance

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Oxacillin resistance among *Staphylococcus aureus* isolated from peritoneal dialysis related peritonitis

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Peritonitis remains as the major cause of peritoneal dialysis (PD) failure. *Staphylococcus* spp. are the main etiologic agents of these infections, and lower resolution rates are associated to *Staphylococcus aureus* infections. Oxacillin resistance is an important marker to correct therapy, since implies in resistance to all other β-lactam antimicrobials. We aimed to evaluate the oxacillin resistance, *mec*A gene and staphylococcal cassette chromosome surrounding *mec*A gene (SCC*mec*) in *S. aureus* isolated from peritonitis in patients under PD, from a single center through 1996 to 2008. Seventy-two *S. aureus* strains were isolated from PD fluid by conventional and standardized methods; clinical signals were associated to determine peritonitis diagnosis. Strains were identified by the macroscopic morphology, Gram stain, and catalase and coagulase production. Oxacillin susceptibility was carried out by minimal inhibitory concentration (MIC) (E-test strips) method and susceptibility breakpoints follow CLSI 2010 (M100-S20). Polymerase chain reaction (PCR) targeting *mec*A gene was carried out, and SCC*mec* type was determined by multiplex-PCR; established quality control strains were included in each one of the tests. There was a tendency to lower *S. aureus* prevalence over time (61 isolates on the early five year period vs. 11 on the second). Of the 72 *S. aureus* isolated, 69 were available to laboratory tests. E-test method detected 10/69 (14.5%) oxacillin-resistant strains (MIC ≥ 4 µg/mL), while *mec*A gene was detected in 8/69 (11.6%). SCC*mec* types were detected in only 3 strains carrying *mec*A gene: 2 strains were classified as SCC*mec* type III and one strain was classified as SCC*mec* type IA; other samples could not be classified with this approach. This study reveals the presence of oxacillin resistant among *Staphylococcus aureus* causing peritonitis in PD patients. To identify a strain with oxacillin resistance mediated by *mec*A gene is ultimate to decide the correct management of the infection, however, it is known that *mec*A can mediated heterogenous resistance to oxacillin, which could difficult this resistant mechanism recognition by phenotypic techniques. Surprisingly, detection of *S. aureus* carrying SCC*mec* types IA and III let us to think that these infections could be acquired on hospital environment, once that these cassette are traditionally associated to nosocomial infections. This conclusion is limited due to the high number of non-classified strains by SCC*mec* multiplex PCR approach. Therefore, results showed herein alert for the presence of *mec*A mediated oxacillin resistance in *S. aureus* causing peritonitis in PD patients and the presence of SCC*mec* types associated to hospital acquired infections.

Acknowledgement: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Keywords Peritonitis; Peritoneal Dialysis; *Staphylococcus aureus*; Oxacillin resistance; *mecA* gene; *SCCmec.*

Patterns of *Helicobacter pylori* Resistance to Metronidazole, Clarithormycin and Amoxicillin in Saudi Arabia

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Background: The work was carried out to study the resistance pattern of *Helicobacter pylori* and its susceptibility to the antimicrobial agents in Saudi Arabia.

Methods: Forty six (46) endoscopic of gastritis or duodenal ulcer were collected at Al-Iman general hospital, Riyadh, and were tested by E test. *H. pylori* ATCC 43504 was used as a reference for quality control. The antimicrobial agents tested against *H. pylori* were, Metronidazole, Clarithromycin and amoxicillin.

Results: Of the isolates tested 69.5% were resistant to Metronidazole (MIC > 8 mg/l), 21% to Clarithromycin (MIC > 1 mg/l) and 11% were multiresistant. No resistance to amoxicillin was observed. Resistance to Metronidazole was more common in female.

Conclusion: the present study demonstrates high metronidazole resistance rate of H.pylori isolates in Saudi Arabia. Regimens containing metronidazole are best avoided. Trials to test other antimicrobial combinations are recommended.

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Penicillin MBIC and MBEC values for biofilm-forming staphylococci isolated from subclinical bovine mastitis

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Biofilm-forming ability is being increasingly recognised as a virulence factor in bovine mastitis staphylococci, contributing for their persistence in the host, the evasion of its defences and survival at high antimicrobial concentrations. Although *Staphylococcus aureus* is a major mastitis pathogen, several coagulase-negative staphylococci, as *Staphylococcus epidermidis,* are frequently isolated microorganism from ruminant mastitis. This disease, hard to eradicate, has an important economic impact to dairy farmers, as antimicrobial therapy is required, rising health care costs, allied to decreased milk quality and production, and increased culling and death rates.

Penicillin G is widely used and frequently recommended as first choice for mastitis treatment. In the present study addressed the effect of minimum biofilm inhibitory concentrations (MBIC) and minimum biofilm eradication concentrations (MBEC) of penicillin G upon biofilm produced by staphylococci isolates from bovine subclinical mastitis. Quantification of biofilm formation by *S. aureus* (n=21) and *S. epidermidis* (n=22) mastitis isolates, previously identified as biofilm-producer, was performed by broth microdilution using a modified microplate Alamar Blue (AB) assay. Bacterial suspensions ($5x10^5$ CFU/ml) in Mueller-Hinton broth were placed in 96-well plates and incubated for 24h at 37ºC to allow for biofilm formation. Then, penicillin concentrations ranging from 2 µg/µl to 8192 µg/µl were added to each well, and plates were again incubated for 24h at 37ºC. Finally, AB was added to each well and absorbance read at 570 nm wave lenght.

MBEC values were higher than MBIC values for all stains under study. For *S. aureus*, MBIC and MBEC values varied ranged from 1024 to ≥ 8192 µg/µl, while for *S. epidermidis* these values varied from ≤128 e ≥ 8192 µg/µl.

Our *in vitro* results suggest that penicillin is not a viable option for erradicating of staphylococci biolfilms, as the penicillin dosis required for achieving antimicrobial concentration values ≥ 8192 µg/µl would pose a potential toxic effect to animal health. The *in vivo* inhibition or erradication of biofilms formed by staphylococci subclinical mastitis isolates is extremely difficult and MBIC and MBEC values should be determined for other antimicrobial drugs, in order to identify the most efficient in the therapy for subclinical bovine mastitis caused by biofilm-producing bacterial strains.

Keywords Biofilm; Staphylococci; subclinical bovine mastitis; Penicillin G; MBIC; MBEC

Phenotypic and genotypic antibiotic susceptibilities of *Campylobacter coli* isolates from ready-to-cook poultry retail samples

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Campylobacter is a leading cause of zoonotic enteric infections in most developed and developing nations (EFSA, 2009; WHO, 2010). This gram-negative spiral bacteria, infects most broilers by the time they reach 4 weeks of age thus making the consumption of fresh poultry a major pathway of human exposure to *Campylobacter* spp.. At multiple steps along the food chain, including production, processing, distribution, retail marketing, and handling or preparation, this pathogen can occur on poultry products by cross contamination.

Due to the often self-limiting diarrhoea in humans, antibiotic treatment is normally not required being nevertheless indicated for severe and prolonged enteritis, septicaemia, and for people at risk such as immune-compromised patients. Nowadays, the most common antimicrobial agents for treating *Campylobacter* spp. infections are fluoroquinolones, such as ciprofloxacin and macrolides, such as erythromycin.

The main purpose of this study was to evaluate the antibiotic susceptibilities of *Campylobacter coli* isolates from ready to cook poultry retail products.

Different processed poultry products, ready to cook and packaged under modified atmosphere (poultry deboned pieces, steaks, fresh sausages, burgers, balls, Stroganoff, hot wings, marinated poultry meat and seasoned poultry meat), from different industrial units, were collected in different days from October to December 2009, at four different points of retail, situated in the Lisbon metropolitan area. Detection of *Campylobacter* spp. was performed according to EN/ISO 10272-1:2006 and isolates were identified by multiplex PCR according to Samosornsuk *et al.* (2007). The majority of the samples were contaminated with *Campylobacter coli*. A total of 65 *C. coli* isolates were attained. The evaluation of resistance to different antibiotics was done using antibiotic susceptibility testing (disk diffusion method) according to the *Comité de l'antibiogramme de la Societé Française de Microbiologie* (2010). Ampicillin (10µL), erythromycin (15 µL), tetracycline (30 µL), gentamycin (10 µL), ciprofloxacin (5 µL), amoxicillin and clavulanic acid (20 + 10 µL) were tested. The presence of the point mutation A2075G present in the 23S rRNA gene, attributed to high-level erythromycin resistance was screened for using PCR-restriction fragment length polymorphism (RFLP-PCR), according to Kurinčič *et al.* (2007). The phenotypic and genotypic data were compared.

Gentamycin showed to be active for most of the isolates with only 6% of them resistant. On the other hand, most of the *C. coli* isolates (91%) were resistant to ciprofloxacin (5 µL) and tetracycline (30 µL). 49% of *C. coli* isolates were resistant to ampicillin (10µL), while 42% of the isolates revealed resistance to erythromycin (15 µL). Finally, 38% were resistance to amoxicillin and clavulanic acid (20 + 10 µL). The results of phenotypically resistant to erythromycin isolates were corroborated by RFLP-PCR, revealing the presence of the point mutation A2075G. It was also found that 13 of the isolates were resistant to erythromycin without possessing the mutation which indicates the presence of an alternative resistance pathway.

The level of multidrug resistant isolates under study was taken in account and was concluded that 5% of *C. coli* showed resistance to all 6 antibiotics. 17% showed resistance to 5 and 4 of the antibiotics used while 26% were resistant respectively to 3 and 2 of the antibiotics.

From these results we can therefore appreciate the need of an effective implementation of safety control management system HACCP- based along the food chain, in order to limit cross contamination with *Campylobacter* spp. and its occurrence in a high frequency. On the other hand, it is worth mentioning once again the consequences of frequent use of antibiotics in primary production as preventive and therapeutic agents which conducts to the development of multidrug resistance strains.

Keywords: retail products, Campylobacter, poultry, antibiotics, multidrug-resistant

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Phenotypic and genotypic characterization of antimicrobial resistance in *Escherichia coli*, *Salmonella* Serovars and *Enterococcus* spp. isolated from animals, abattoirs and retail meat.

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Introduction: Excessive antimicrobial use during livestock production is recognized as a major contributing factor to the emergence of antimicrobial-resistant bacteria. Studies have suggested that the antimicrobial-resistant pathogenic *E. coli* and *Salmonella* strains infecting humans originated from animals. Therefore presence of resistant bacteria in foods of animal origin may pose a human health risk. **Objective:** The aim of this research was to phenotypically and genotypically characterize antimicrobial resistance (AMR) in *E. coli*, *Enterococcus* spp. and *Salmonella* serovars isolated from animals, during animal slaughter and processing and from retail meats. **Methods:** Commensal *E. coli*, enterococci and *Salmonella* serovars were isolated from samples taken from commercial beef and pork processing plants and from retail meats (poultry, pork, and beef). Pathogenic *E. coli* O157:H7 was isolated from fecal and oral samples collected from beef cattle in feedlots. The susceptibility of *E. coli*, *Salmonella* serovars (to a panel of 15 antimicrobials) and *Enterococcus* spp. (to a panel of 17 antimicrobials) was determined using a microbroth dilution method with a sensititre system® (Trek Diagnostics, Ohio, USA) and results were interpreted according to the criteria of the Clinical and Laboratory Standard Institute guidelines (CLSI, 2010). PCR was used for the detection of following resistance genes: tetracycline *tet*(A), *tet*(B), *tet*(C), sulfonamides (*sul1, 2, 3*), β-lactamase (bla_{CMY-2}, bla_{SHV}, bla_{TEM}, bla_{CTX-M}), aminoglycosides (*aac3(IV), aadA/B, strA/B*) in *E. coli* and *Salmonella* isolates. A microarray assay was used to detect 20 species specific, 18 virulence and 170 AMR genes in *Enterococcus* spp. Data was analyzed in order to determine the prevalence of AMR and AMR genes; statistical associations among AMR pheno- and genotypes and among various sample types. **Results:** The majority of *E. coli*, *Enterococcus* spp. and S*almonella* serovars isolated from retail chicken samples showed resistance to various antimicrobials whereas AMR was lower in isolates from retail beef and pork. The AMR genes for tetracycline, sulfonamide, macrolides and aminoglycosides were commonly found in resistant isolates and significant statistical association were found among AMR pheno-and genotypes and among AMR genotypes. About 60% of *E. coli* O157:H7 from commercial feedlots were susceptible to all tested antimicrobials however, resistance to tetracycline and sulfonamide was common. Antimicrobial resistance in *E. coli* and *Enterococcus* spp. isolated from beef processing plants was higher in samples from equipments used for meat processing. The *tet*, *sul* and *str* genes were also highly prevalent in *E. coli* from those samples. The majority of *E. coli* from the pork processing plant were susceptible to the tested antimicrobials but resistance to ampicillin (26% isolates), streptomycin (30%isolates) and tetracycline (55% isolates) was found. β-lactamase genes bla_{CMY-2}, bla_{TEM}, bla_{CTX-M} were found in number of *E. coli*. This presentation will discuss data in detail about the AMR and genetics of AMR throughout the animal-food chain. **Significance:** The data of this research would help understanding the dynamics of AMR in animals, during meat processing and in retail meats and allow regulatory authorities to for promoting prudent use of antimicrobial during animal production.

Keywords: Antimicrobial resistance (AMR), *Escherichia coli*, *Salmonella, Enterococcus* spp. AMR genes, meat, animal, meat plant

Prevalence and Antibiotic Resistance of *Salmonella* spp. Isolated from Retail Raw Meats in Korea

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Salmonella spp. is one of the most common causative agents of foodborne illness in Korea. Raw and processed meat products, including poultry, beef, and pork, are the principal reservoir of *Salmonella*. In addition, the antimicrobial resistance in *Salmonella* leads to a public health risk as it may potentially affect the efficacy of drug treatment in humans. The objective of this study was to investigate the prevalence and the antibiotic resistance patterns of *Salmonella* spp. isolated from retail raw meats. Beef (n=80), pork (n=80), and chicken (n=56) meats purchased from traditional markets, department stores, wholesales markets, and retail markets in Seoul were used. Twenty-five grams of samples were pre-enriched in 225ml of buffered peptone water, at 37℃ for 24h. For selective enrichment, pre-enriched samples (each 0.1mL) were inoculated into 10 mL of Rappaport-Vassiliadis broth followed by incubating at 42℃ for 24h. Enriched samples were streaked onto xylose lysine desoxycholate agar and then incubated at 37℃ for 24h. Suspected colonies (round and red colonies with black center) were subcultured on nutrient agar at 37℃ for 24h for confirmation with VITEK2 using gram negative test kits. To determine the antibiotic resistance patterns of isolates, disc diffusion method using 16 antibiotics was performed in accordance with National Committee for Clinical Laboratory Standard (NCCLS). The prevalence of *Salmonella* was 12 % in retail meats (26 out of 216 samples). The chicken meats were highly contaminated with *Salmonella,* 26.8 % of chicken followed by 8.6% of pork, and 5% of beef. Most isolates were resistant to streptomycin (11 out of 26 isolates, 42.3 %) followed by chloramphenicol (7 out of 26 isolates, 26.9 %), tetracycline (5 out of 26 isolates, 19.2 %), cefotaxime (4 out of 26 isolates, 15.3 %), ampicillin and gentamycin (3 out of 26 isolates, 11.5 %), cephalothin (2 out of 26 isolates, 7.7 %), cefazolin and cefoxitin and trimethopenem/sulfamethoxazole (1 out of 26, 3.8 %). No isolate was resistant to amikacin, amoxicillin/clavulanic acid, cefepime, ciprofloxacin, norfloxacin, and imipenem. Nine isolates of *Salmonella* (34.6 %) were multi-drug resistant, resistance to more than three drugs (5 isolates from chicken meats, 3 isolates from pork, and 1 isolate from beef). This study showed the prevalence and antibiotic resistance patterns of *Salmonella* isolated from meat samples. The meat products contaminated by *Salmonella* have the possibility to give the serious risk for human health. Therefore, to prevent food borne salmonellosis, surveillance and monitoring of animal products will provide important epidemiological data for public health

Keywords *Salmonella*, prevalence, antibiotic resistance

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Prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from Beef, Pork and Chicken meat in Republic of Korea

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In many countries, *Staphylococcus aureus (S.aureus)* is a cause of many infectious diseases to human and anmal and the third common pathogen causing outbreaks of food poisoning in Korea. Antibiotic resistance strains of *Staphylococcus spp*. are distributed worldwidely, and these strains cause medication failure in clinical cases. The aims of this study was to determine the prevalence and antibiotic resistance of *S.aureus* in retail raw meat. The beef (n=80), pork (n=80) and chicken (n=80) meat were collected from meat shop in department stores, supermarkets, and traditional market in Seoul, Korea between 2009 and 2010. All samples (25 g of each sample) were added to TSB with 10% NaCl (225ml/sample) and incubated at 36°C for 24h. The cultured broth were streaked onto baird parker media with egg yolk tellulite and incubated at 36°C for 24h. Two suspicious colonies were plated on nutrient agar followed by coagulase confirmation test and colony PCR analysis or VITEK2TM. Antibiotic susceptibility tests were performed by disk diffusion method, and methicillin-resistant *S.aureus* (MRSA) was identified by minimal dilution method to determin oxacillin susceptibility in accordance with National Committee for Clinical Laboratory Standard (NCCLS). *S.aureus* were isolated from 26.6 % (64 of 240) of all samples ; 22.5 % (18 of 80) in pork, 22.5 % (18 of 80) in beef, and 32.5 % (26 of 80) in chicken. Seventyfive precent of isolated *S.aureus* were resistance to at least one antibiotic, and 19 % of S.aureus strains was resistance to more than three antibiotics. The antibiotic resistance of isolated *S.aureus* were 64 % to penicillin, 28 % to tetracycline, 25 % to erythromycin, 16% to amikacin, 16% to clindamycin, 6 % to oxacillin and 2 % to trimethoprim/sulfamethxazole. There were no vancomycin-resistance *S. aureus* (VRSA), but four strains (6.25%) were MRSA. Minimal inhibition concentration (MIC) of each MRSA strains were 8, 8, 16 and 32 μg/ml of oxacillin, respectively. This data showed high prevalence and antibiotic resistance rate of *S.aureus* isolated from retail raw meat. Therefore, meat products contaminated with *S.aureus* have the possibility to present the serious risk for human health. It indicates that it is required to prevent *S.aureus* contamination in raw meat and to monitor antibiotic resistance continuously.

Keywords *S.aureus*, Antibiotic resistance, MRSA, prevalence

Prevalence and antimicrobial susceptibility of *Escherichia coli* isolated from retail meat products

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Meat and meat products are an important source of food borne infection and the most important link between food-producing animals and humans. Antimicrobial agents are widely used for the treatment of animals and the concern on antimicrobial susceptibility bacteria is gradually increased worldwide. The objective of this study was to investigate the prevalence and antimicrobial susceptibility of *E. coli* contaminated in beef, pork, chicken and sashimi. All 248 samples (beef, pork, chicken, and sashimi) were purchased from meat shop in department stores, supermarkets, and traditional marketplaces in Seoul, Korea from 2009 to 2010. For the enrichment of the *E.coli*, 225ml EC broth was added to 25g of each sample, and then incubated at 37oC for 24h. The enriched cultures were streaked onto EMB agar and incubated at 37oC for 24h. The suspicious colonies were transferred on nutrient agar and incubated at 37oC for 24h. After incubation, *E.coli* performed the biochemical confirmation test with VITEK2. All isolates were tested for the sensitivity to antibiotics by the disc diffusion method according to the National Committee for Clinical Laboratory Standard (NCCLS). In addition, shiga toxin producing tests using the multiplex real-time PCR (*stx1*and *stx2*). *E.coli* isolates were obtained from beef, pork, chicken and sashimi resulting in an overall isolation rate of 41.5% (103/248). Of these isolates, 20 were obtained from 56 beef samples (35.7%), 29 of 80 pork samples (36.3%), 53 of 80 chicken samples (66.3%) and 1 of 16 fish samples (6%). Two isolates possessed shiga toxin gene (stx 2). The most frequently observed resistance in *E. coli* isolates was to tetracycline (TE 40%), followed by resistance to streptomycin (S, 32.2%) and ampicillin (AMP, 31.6%). Of the isolates, 86.5% of *E. coli* strains showed resistances against more than three of antimicrobial agents tested. The results of this study indicate that the raw meats were most commonly contaminated with *E.coli*. In addition, meats are possible to infection of not only multiply antibiotic susceptibility but pathogenic *E.coli* strains. Therefore further studies are needed to control and prevent of E.coli infection.

Keywords : *Escherichia coli*, antimicrobial susceptibility, retail meat product

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Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in a Tertiary-care Children Hospital in Central Nepal

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Nosocomial infection is a major problem in the world today. Methicillin Resistant *Staphylococcus aureus* (MRSA) strains shows a particular ability to spread in hospitals and is one of the greatest challenges for modern antimicrobial therapy in many countries, particularly because of the multidrug resistance.

The present study was carried out with the aim to find out the prevalence of MRSA infections and their antimicrobial susceptibility pattern in pediatric patients attending a tertiary-care referral hospital located in Central Nepal.

Various clinical samples collected from patients were inoculated to Mannitol Salt Agar and incubated at 37^{o}C for 24 hours. Identification of *Staphylococcus aureus* was confirmed by Gram positive cocci in clusters, mannitol fermenting colonies, catalase positivity, coagulase positivity and DNase positivity. Antimicrobial susceptibility testing was also performed by Kirby-Bauer disc diffusion method. Interpretation criteria were those of the national committee for clinical laboratory standards.

Of the total 210 clinical samples, *S. aureus* was isolated in 30.95 % (n=65) cases. Among the *S. aureus* isolates, 29.23 % (n=19) were found to be methicillin resistant. More than sixty eight percent isolates of MRSA were from inpatient departments while 31.57 % were from outdoor patients. All isolates of MRSA showed resistant to ampicillin and cloxacillin. More than 90 % of MRSA were found to be resistant to cotrimoxazole, while less than 50% of MRSA were resistant to tetracycline and ciprofloxacin. However, none of the MRSA strains were resistant to vancomycin.

Vancomycin seems to be the only antimicrobial agent which showed 100% sensitivity and so may be used as the drug of choice for treating multidrug resistant MRSA infections. However, regular monitoring of vancomycin sensitivity should be carried out.

To reduce the prevalence of MRSA, the regular surveillance of hospital acquired infection including monitoring of antimicrobial (especially vancomycin and other newer glycopeptides) susceptibility pattern of MRSA and formulation of definite antimicrobial policy may be helpful.

Keywords: Central Nepal; MRSA; resistant

Resistance against antibiotic peptides. Molecular bases of colistin (polymyxin E) resistance in *Acinetobacter baumannii*.

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In the last years, the Gram negative cocobacillus *Acinetobacter baumannii* has rapidly increased its relevance as a serious nosocomial threat, mostly as an opportunistic pathogen in ICU wards (1). This is due both to its capacity to thrive under a variety of harsh environments and conditions; as well as to its outstanding capacity to develop resistance to antibiotics under current clinical use. Nowadays, an increasing incidence of multi- and even pan-resistant isolates is reported worldwide, in many cases associated with outbreaks of high morbidity and mortality. This has led to the use to polymyxins, mostly polymyxin E (colistin, PXE) as a last resort (2), despite its contrasted toxicity and poor tissue penetrability.

Unfortunately, colistin-resistance isolates have been sporadically leaking out throughout the last decade (3). For other Enterobacteriacea, polymyxin resistance was intrinsically associated to changes in LPS structure with a resulting decreased anionicity (4). In contrast to the rapid dissemination of resistance against other antibiotics in *A. baumannii*, outbreaks of PX-resistant isolates were mostly confined into its original location. This unusual behaviour prompted us to get a deeper insight into the molecular basis underlying this process.

We have obtained in vitro a PXE resistant strain from the parental ATCC 19606, by growth under increasing pressure of the antibiotic with a final resistance factor of 16. As expected, PXE-resistant isolate coursed with lower binding of colistin and strong restructuration of its outer membrane. The most appealing conclusion is the high cost to maintain this resistance. The PXE-resistant bacteria showed an increased generation time (62 vs 40 min), high instability reverting rapidly to the susceptible phenotype when grown in absence of PX, and impaired fitness, in agreement with complementary proteomic and metabolic data. Additionally, the resistant strain showed a crippled capacity to form biofilm and reduced virulence against *Dictyostelium* model. These characteristics may account for the reduced dissemination of the resistant strain and suggest alternative targets for a combination therapy aimed to prevent colistin resistance.

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Keywords: polymyxin resistance, fitness, *Acinetobacter baumannii* , fitness

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Resistance Detection and Susceptibility Profile in *Staphylococcus* spp. Isolated from Patients with Urinary Tract Infection

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Urinary tract infection (UTI) is one of the most frequent infectious diseases in clinical practice. It is also the second most common infection in humans as it is only less prevalent than that in the respiratory tract. *Staphylococcus saprophyticus* is the second most frequent agent of community-acquired UTI, and it is mainly isolated from the urine of sexually active young women. There are also reports of septicemia and pyelonephritis by such organism. This study aimed at evaluating the profile and susceptibility detection in *Staphylococcus* spp. isolated from patients with UTI. Samples with a colony count $\geq 10^5$ CFU/mL by using the Kass method as reference were considered to be compatible with UTI. Drug susceptibility testing was performed by disk diffusion using oxacillin, cefoxitin, cephalotin, gentamicin, linezolid, norfloxacin, penicillin G, sulfamethoxazole/trimethoprim, vancomycin, nitrofurantoin, amoxicillin/clavulanic acid, erythromycin and clindamycin. Oxacillin resistance was determined by the disk diffusion method and detection of the *mecA* gene. The disk approximation (D-test) method was used for detection of induced resistance to antimicrobials belonging to the MSL group (macrolides; lincosamines and streptogramins). Among the 100 staphylococcus samples studied, the following were identified: 57 *S. saprophyticus*, 14 *S. epidermidis*, 7 *S. haemolyticus* and 6 *S. warneri*. The resistance percents for *Staphylococcus* spp. were: Oxacillin 78.0%, Penicillin 73.0%, Erythromycin 39.0%, Cefoxitin 27.0%, Sulfamethoxazole/trimethoprim 18.0%, Norfloxacin 14.0%, Gentamicin 14.0%, Amoxicillin/clavulanic acid 10.0%, Clindamycin 7.0%, Cephalotin 5.0%. All the samples were susceptible to vancomycin, linezolid and Nitrofurantoin. Among the studied samples, 18 *mecA*-positive staphylococci were found. These were 6 *S. epidermidis*, of which 3 had type-III *SCCmec* and 3 type-IV; 4 *S. aureus*, 2 type III, one type II and one untypable; 4 *S. haemolyticus*, 2 type II and 2 untypable; 2 *S. saprophyticus*, both type IV; and 2 *S. warneri*, of which one was type III and one was untypable. As regards the tests for oxacillin resistance detection, of the 18 *mecA*-positive staphylococci, 15 (83.3%) were resistant to the cefoxitin and oxacillin disks concomitantly. Of the 57 *S. saprophyticus* studied, 7 (12.3%) were resistant to the cefoxitin disks and none showed the *mecA* gene, whereas 56 (98.2%) showed resistance to the oxacillin disk, and only 2 showed the *mecA* gene. The D-test was positive in 10 isolates (6 *S. saprophyticus*, 3 *S. aureus* and in one *S. haemolyticus*). Clindamycin is an antimicrobial that can be occasionally used for UTI treatment; therefore, in these cases, the non-detection of this resistance type can lead to therapeutic failure. Nitrofurantoin is a specific antimicrobial for the urinary tract, with few collateral effects, low toxicity and low cost, emerging in our study as a therapeutic option against multiresistant *Staphylococcus* spp., which were susceptible only to vancomycin and linezolid among all antimicrobials tested. Although the infections caused *S. saprophyticus* are well documented, the dissemination of these species and antimicrobial resistance have not been thoroughly studied. Despite the fact that they are susceptible to most antimicrobials used, increased resistance and the presence of the *mecA* gene seem to be occurring in these species. The prevalence of *mecA* among the *S. saprophyticus* found in our study was of 3.5%, both of type IV; however, 98.2% showed resistance to the oxacillin disk. The results showed that, although the cefoxitin disk and the *mecA* gene detection were more sensitive for oxacillin resistance detection in different staphylococci species, for *S. saprophyticus* the oxacillin disk showed better results. This probably occurred due to β-lactamase hyperproduction, alteration of another PBP that is not PBP2a, or also confirming the finding by other authors that the cutoff point preconized by CLSI (Clinical and Laboratory Standards Institute) may overestimate oxacillin resistance in *S. saprophyticus*, thus indicating that some changes are necessary for detecting oxacillin resistance in this species.

Keywords: Urinary tract infection; *S. saprophyticus*; Resistance; Antimicrobials.

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Resistance Distribution Profile of MBL, ESBL and Multi-Drug Resistant Gram-Negatives Isolated at a Tertiary Care Hospital in India

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Prevalence of extended spectrum β-lactamase (ESBLs) and metallo- β -lactamase (MBLs) constitute a serious threat to current β–lactam therapy leading to treatment failure and consequent escalation of cost. In a tertiary care hospital the patients are admitted for causes other than the underlying infection. Detection of infection and subsequent identification of resistance in the infectious agent puts additional burden of investigating effective drug for cure. To ease the process of drug selection to treat MBL and ESBL isolates a detailed study was initiated to identify the resistance distribution pattern in isolates. Antibiogram profiles were determined to commonly used antibiotics and confirmation of ESBLs production was carried out by the disk diffusion assay using ceftazidime in the presence and absence of clavulanic acid. Metallo β-lactamase producers were confirmed using Imipenem and EDTA disks. A total of 390 Gram negative isolates were grouped into four categories sensitive 89 (22.83%), ESBL producers 137 (35.12%), MBL producers 37 (9.48%) and multidrug resistant 127 (32.56%). There were 174 *E. coli*, 71 *Pseudomonas aeruginosa*, 73 *Klebsiella pneumoniae*, 47 *Klebsiella* spp and 26 *Acinetobacter baumanii*. Of the 137 ESBL producers, 84 (61.31%) were E. coli, 23 (16.78%) were *K. pneumoniae*, 18 (13.13%) were *Klebsiella* spp, 11 (8.02%) *P. aeruginosa* and 1 (0.72%) was *A. baumanii*. Of these 137 ESBLs all but one were sensitive to Imipenem, 128 (93.43%) were sensitive to cefoperazone+sulbactum and 119 (86.86%) were sensitive to piperacillin+tazobactum. The lone imipenem resistant ESBL producer was also found to be MBL producer and it was a *K. pneumoniae*. Of the 37 MBL producing isolates 18 (48.65%) were *P. aeruginosa*, 11 (29.72%) were *A. baumanii*, 7 (18.92%) were *Klebsiella pneumoniae* and 1 (2.7%) each of E. coli and *Klebsiella* spp. Of these 37 MBL producers 13 were sentive to meropenem, 6 to cefoperazone+sulbactum, 7 to piperacillin+tazobactum and 25 to polymyxin-B. Similarly, in the 127 (32.56) multi-drug resistant isolates 37 (29.13%) were E. coli, 31 (24.40%) were *K. pneumoniae*, 31 (24.40%) were *P. aeruginosa*, 15 (11.81%) Klebsiella spp., and 13 (10.23%) were *A. baumanii*. All the 127 multidrug resistant isolates were sensitive to Imipenem, 61 (48.03%) to cefoperazone+sulbactum and 65 (51.18%) to piperacillin+tazobactum. Our results indicate that the majority of ESBLs were amenable to Imipenem, cefoperazone+sulbactum and piperacillin+tazobactum but MBL producers were largely resistant to the above group of antibiotics. About two-third MBL producers, however, were amenable to polymyxin-B. Also, all the multi-drug resistants were amenable to imipenem and about 50% to piperacillin+tazobactum and cefoperazone+sulbactum. Thus, imipenem appears to cover most non MBL producing isolates even after its introduction in 1980. So far there is only one isolate reported here of a combined producer of MBL and ESBL and therefore not amenable to imipenem.

Keywords ESBL; MBL; Multidrug resistance; Imipenem Sensitivity

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Resistance Profile of *Staphylococcus aureus* Resistant to Methicillin - MRSA Isolated From Patients in a Brazilian Teaching Hospital

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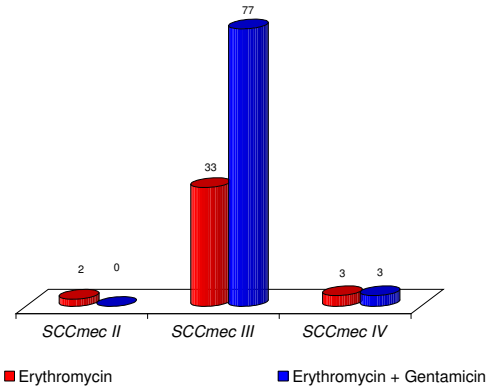
Occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) is receiving greater attention because of the sudden increase in morbidity and mortality due to nosocomial infection outbreaks. This study aimed to analyze the resistance profile of strains of MRSA isolated from clinical and surveillance cultures of patients at a Brazilian teaching hospital. A total of 384 *S. aureus* strains isolated from surveillance cultures, secretions and blood cultures of 170 patients seen at Hospital Estadual Bauru (HEB), Brazil, were identified and tested regarding their susceptibility to oxacillin (1 µg) and cefoxitin (30 µg), as well as to vancomycin (30 µg), erythromycin (15 µg), and gentamicin (10 µg). In contrast, genotyping was only performed for the first sample of each patient and, in some cases, when a blood culture and surveillance culture were available during the same period. The genotypic resistance profiles were analyzed in 229 samples using the PCR method to investigate the presence of the *mecA* gene, the positive one's were subjected staphylococcal cassette chromosome subtyping (SCC*mec*) by PCR-Multiplex. Of the 384 samples of *S. aureus*, 270 (70.3%) were resistant to oxacillin and 274 (71.3%) to cefoxitin by disk diffusion method. The phenotype of simultaneous resistance to gentamicin and erythromycin was observed in 63.1% of samples with MRSA phenotype and only 1.8% of the samples with MSSA phenotype. None of the samples included in this study was resistant to vancomycin. The analysis of the resistance by detection of *mecA* gene in 292 *S. aureus* strains showed that 167 (72.9%) were MRSA, while 62 (27.1%) were sensitive. Of the 167 *mecA* positive 142 (85%) and 143 (86%) showed phenotypic resistance to oxacillin and cefoxitin, respectively. The characterization of the SCC*mec* showed 157 samples (94%) had SCC*mec* type III or variations of the Type III, 7 samples (4.2%) had SCC*mec* type IV and 3 (1.8%) type II SCC*mec*. Of the 157 samples SCC*mec* type III or variations, 33 samples (21%) showed phenotype of resistance to erythromycin and 77 samples (49%) were resistant to gentamicin and erythromycin concomitantly. Analysis of the resistance phenotypes of the 7 SCC*mec* type IV MRSA isolates showed that 3 (42.8%) were resistant to erythromycin, 3 (42.8%) were resistant to gentamicin and erythromycin and one sample showed sensitivity to all antibiotics tested. In our study the majority of MRSA samples were SCC*mec* type III or variations. The analysis of the resistance profile of these samples is important because the spread of resistant strains resistant to antibiotics most used in clinical practice may be a limiting factor for the treatment of staphylococcal infections.



Keywords *Staphylococcus aureus*; MRSA; resistance; SCC*mec*
Financial support: FAPESP, CNPq.

RNase H- determination of its role in mycobacteria.

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Tuberculosis (TB) together with AIDS are amongst the leading infectious causes of death worldwide. In fact, TB is a major cause of death among people living with HIV/AIDS and HIV is the main reason for failure of a TB control plan. Currently applied chemotherapy for both of the diseases is far from satisfactory.

One of the branches of anti-HIV research focuses on finding inhibitors for viral RNase H, a domain of viral reverse transcriptase. RNase H, responsible for removing RNA from RNA/DNA hybrids, is ubiquitous in different organisms, including mycobacteria. Its role in DNA replication is already well established; it removes RNA primers from the DNA strand. Some studies suggest that RNase H might also be involved in DNA repair. We decided to test whether inactivation of RNase H gene of mycobacteria would affect their fitness. Therefore we used the technique of gene replacement based on the process of homologous recombination and obtained mutants of *M. smegmatis* lacking functional copies of genes encoding RNase HI, RNase HII and RNase HI/RNase HII. All of the mutants are viable during normal growth conditions, therefore there must be an alternative mechanism for removing primers during replication. Future plans include testing if mycobacterial RNase H could be involved in DNA repair by monitoring viability of mutants and complemented mutants under different stresses (UV, H_2O_2, mitomycin C, inhibitors of HIV RNase H).

Keywords RNase H, Mycobacterium, HIV, gene replacement, DNA repair

Sensibility and Resistance of *Staphylococcus aureus* from Cows with Subclinical Mastitis and from Milking Machine Environment in a Brazil Dairy Property

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The milk must be free of contamination to be considered a favorable environment for multiplication and vehicle of various pathogens such as *Staphylococcus* spp, which are microorganism, mostly producing coagulase, standing out among them *Staphylococcus aureus*, which substrate when present in food, has the ability to synthesize enterotoxins that cause food poisoning and a series of infections. The objective of this study was to evaluate the sensibility and resistance profile of strains of *S. aureus* from milk of cows with subclinical mastitis and the environment in a milking dairy farm in Brazil. During the period August 2008 to July 2009 were collected milk samples from cows reacting to the California Mastitis Test (CMT), on a farm in the region of Indianapolis-MG. The samples of milk were being made monthly milk samples placed in sterile tubes with screw aseptically and stored in coolers with ice. Were made, yet, swabs of teatcup liners, hoses conductive milk, expansion tank and hands of employees and which were placed in tubes containing BHI sterile screw. Were did phenotypic identification of *S. aureus* and soon after the procedures to evaluate the sensibility and resistance of *S. aureus* antimicrobials were performed according to the protocol of NCCLS (2000), using Mueller Hinton agar (MH).The antimicrobials tested were: cefepime (30µg), ciprofloxaxin (5µg), chloramphenicol (30µg), clindamycin (2µg), erytromycin (15µg), gentamicin (10µg), oxacillin (1µg), penicillin (10 Un), rifampicim (30µg), sulfazotrim (25µg), tetraciclin (30µg), vancomicim (30µg). We isolated 440 strains of *S. aureus* that showed greater resistance against penicillin (98%), clindamycin (74%), erythromycin (71%), oxacillin (50%), tetracycline (43%), vancomycin (41%) followed by rifampicin (41%) gentamicin (40%), ciprofloxacin (39%), chloramphenicol (25%), cefepime (29%) and sulfazotrim (20%). High rates of resistance of *S. aureus* antimicrobials demonstrate the necessity of performing susceptibility testing in vitro, in order to inhibit the indiscriminate use of these products, so as to minimize the emergence of resistance of these microorganisms and thus reduce the occurrence of antimicrobial residues in milk

Keywords: antimicrobials; milk; microorganisms; *Staphylococcus aureus*;

Serotype distribution and antibiotic susceptibility patterns of invasive *Streptococcus pneumoniae* circulating in Tunisia

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To investigate serotype distribution and antimicrobial susceptibility patterns of invasive *S. pneumoniae*, 134 clinical strains obtained from invasive sites were collected from three Tunisian universities hospitals, between 2000 and 2008. Forty five isolates were from children and 89 from adults. Seventy seven strains were isolated from pleural fluids, 29 from cerebrospinal fluids and 28 from blood.

They were identified on their morphologic and metabolic characters. Antibiotic susceptibility was done using disk diffusion method. MICs were determined by E-test for 3 β-lactams: penicillin G, amoxicillin and cefotaxime. Pneumococcal serotyping was done by multiplex PCR.

The most frequent serotypes were 23F, 19F, 6B, 14 and 6A among children, and 19F, 19A, 6A, 6B and 24F among adults. The potential coverage by 7 and 23-valent pneumococcal conjugate vaccines were 60% and 80% in children and 38.2% and 60.6 % in adults, respectively. Fifty percent of isolates were susceptible to penicillin (PSP) (MICs range: 0.008 - 0.094 µg/ml), 32% were intermediate (PIP) (MICs range: 0.125 - 1 µg/ml) and 18% had high resistance level (PRP) (MICs range: 2 - > 32 µg/ml). PIP and PRP were also resistant to erythromycin (72%), tetracyclines (47%), trimethoprim-sulfamethoxazole (34.5%) and chloramphenicol (14.5%). In all ages, the predominant serotypes, except serotype 6A, exhibited high rates of resistance to penicillin (from 44.4 % in serotype 6B to 73.5% in serotype 19F). The less commonly isolated serotypes (35F, 4, 1, 34, 10A, 15A, 33F, 17F and 3F) were PSP.

The surprisingly high level of antimicrobial resistance among invasive *S pneumoniae* considerably strengthens the potential impact of a childhood pneumoccal vaccination in Tunisia.

Keywords: *Streptococcus pneumoniae,* invasive, serotypes, antimicrobial resistance

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Staphylococus aureus carrying SCC*mec* IV: evaluation of antimicrobial resistance of different STs isolated from hospitals in Rio de Janeiro, Brazil

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Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have been described globally. MRSA carrying the staphylococcal cassette chromosome *mec* (SCC*mec*) IV in hospitals generates great concern, since these isolates appear to exhibit a better balance between resistance and virulence, demonstrating adaptive advantages. The aim of this study was to evaluate the antimicrobial resistance profiles in MRSA SCC*mec* IV from different pulsotypes and sequence types (ST) isolated in seven hospitals in Rio de Janeiro. Minimal Inhibitory Concentrations (MIC) to ciprofloxacin, clindamycin, chloramphenicol, erythromycin, gentamicin, oxacillin, rifampicin, trimethoprim/sulfamethoxazole, tetracycline and vancomycin by the broth microdilution method were determined for 28 isolates, of which 21 were classified into ST 1(7 isolates), 5 (11) and 30 (3). PCR method was also used to detect the erythromycin resistance genes (*erm*A, *erm*B, *erm*C). All isolates were sensitive to vancomycin, trimethoprim/sulfamethoxazole and tetracycline, with MICs lower than 1, 9,5/0,5 and 2 µg/mL, respectively. Four (13.8%) isolates showed MIC of susceptibility (1 or 2 µg/mL) for oxacillin. Six isolates from ST1 (clonality similar to USA 400) presented MICs of resistance higher than 16, 128, 32 and 64µg/mL for ciprofloxacin, chloramphenicol, clindamicin and erythromycin, respectively. Isolates with clonality similar to USA 800 (ST 5) presented resistance mainly to erythromycin and chloramphenicol (MICs greater than 8 and 32µg/mL). PCR results showed that 71% of the isolates with clonality similar to USA 400 carried only the *erm*C gene, while 72% of the isolates similar to USA 800 carried *erm*A + *erm*C genes. We can conclude that resistance in MRSA type IV isolates from ST1 and ST5 is greater than that observed for ST30. This fact can be related to their prevalence in Rio de Janeiro hospitals.

Keywords: *S. aureus*, SCC*mec* IV, Sequence types, antimicrobial resistance, MICs

Study of Genetic Evolution in *Mycobacterium tuberculosis* isolates from Patient with Active Pulmonary Tuberculosis in the Iranian and Belarusian Isolate

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Background: This is the new comparative geogenetic biodiversity study of M. tuberculosis in Iran and Belarus. Thus, we researched the genetic patterns of strains isolated in the first survey of anti-tuberculosis drug-resistance by rpoB gene as part of the Global Project of Anti-tuberculosis Drug Resistance Surveillance (REIM, IAU).

Design. A 411-bp fragment of the *rpo*B gene, containing the sequence of the 81-bp *rpo*B fragment, was amplified by PCR and the rpoB gene fragments of tuberculosis strains were sequenced using the Amersham auto sequencer. For analysing tree evolution used method UPGMA and Neighbour-Joining. Clinical isolates (70/473) were analyzed by using sequencing gene rpoB and genotyped by program MEGA.

Results: The results were compared with the international database. Multi drug resistant (MDR) was 92% in never treated patients and 8% in previously treated patients. Mutations in rpoB gene and katG genes were detected in 95% and 84% of the MDR strains, respectively. Two clusters were found to be identical by the four different analysis methods, presumably representing cases of recent transmission of MDR tuberculosis. The other strains are divided into 2 groups: group A – similar to the standard and Eastern strains (China, Taiwan) and group B – strains of another genotype. They are grouped separately on the dendrogram and became prevalent in Iran (they are called Iranian residential strains).

Conclusion: This study gives a first overview of the M. tuberculosis strains circulating in Iran during the first survey of anti-tuberculosis drug-resistance. It may aid in the creation of a national database that will be a valuable support for further studies.

Keyword: Genetic evolution, rpoB gene, M. tuberculosis, Iranian and Belarusian isolates

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Study on selection and transfer of antimicrobial resistant *Escherichia coli* from broiler breeders to their progeny

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Escherichia coli is a normal inhabitant of the intestinal tract of chickens and is harmless as long as its growth and colonization is inhibited by other commensal intestinal microbial populations. When an imbalance in bacterial flora of the intestinal tract occurs, *E. coli* may grow and cause extraintestinal infections. *E. coli* disease outbreaks, manifested as septicaemia in both parent flocks and their progeny, are often due to clonal strains which can acquire multiple antibiotic resistance characters along the antimicrobial therapy and eventually transfer it to other intestinal bacteria circulating between birds. The aim of this study was to evaluate the transmission of *E. coli* clones and their antimicrobial resistant characters from broiler breeders to their progeny in absence or presence of an ongoing *E. coli* infection. Three groups of chicks (i.e., A1, B1, C1) characterised by omphalitis symptoms and originated from the same group of broiler breeders (R1) were chosen during the first week of life along with three groups (i.e., A2, B2, C2) of healthy chicks originated from the same group of broiler breeders (R2). Among each group 5-10 animals were picked out. Consistently from each group of birds 5-10 animals at 21 days and at the end of the productive cycle were selected. In particular, for sick birds (A1, B1, C1) 5-10 animals were collected before and 5-10 animals after the antimicrobial therapy at 21 days. In parallel, three pools of environmental dust were sampled. Two weeks after the collection of healthy and sick chicks, 5-10 animals of each of the corresponding broiler breeder group (R1 or R2) were also selected. All selected animals were humanely euthanized and from each of them different organs (i.e., liver, spleen, intestine, heart, lungs and tracheal mucus) were collected and tested for the presence of *E. coli*. In brief, 10 g of dust or sampled organ were diluted 1:10 in Ringer's Solution (Oxoid, Hampshire, UK). An aliquot was plated on Eosin Methylene Blue agar (EMB) and incubated at 37 °C for 24h in duplicate. From each plate, two colonies showing characteristic *E. coli* morphology were selected for confirmation through biochemical tests. *E. coli* isolates were molecular characterised by Pulsed Field Gel Electrophoresis (PFGE), using the standard PulseNet protocol with *Xba*I as restriction enzyme, and automated ribotyping according to the manufacturer instructions with *Eco*RI as restriction enzyme (Oxoid, Hampshire, UK). The antimicrobial sensibility of *E. coli* isolates toward amoxicillin, tetracycline, spectinomycin, enrofloxacin and ciprofloxacin was investigated by the microdilution method following standard procedures reported in the document M31-A2 of the Clinical Laboratory Standard Institute (CLSI). Results on the selection and transfer of antimicrobial resistant *E. coli* isolates from broiler breeders to their progeny will be discussed at the conference.

Keywords antimicrobial resistant *E. coli*; selection and transfer; poultry

Surveillance of antibiotic resistance of *Pseudomonas aeruginosa* nosocomial isolates in the largest University Hospital of Lithuania

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The aim of our study was to estimate the dynamics of resistance rates of *Pseudomonas aeruginosa* (*P. aeruginosa)* strains isolated from patients in Intensive care units of Kaunas Hospital of University of Medicine, the largest university hospital of Lithuania, by testing of current resistance level in year 2008 and comparing it with year 2003, which provides a baseline for reference as resistance continues to progress.

Materials and methods

Isolates were identified with the Phoenix ID system (Becton Dickinson, USA). Antimicrobial susceptibility of all the isolates was tested by the Kirby-Bauer disc diffusion method. The prevalence of multidrug resistant (MDR) strains were investigated among isolates of *P. aeruginosa* tested with piperacillin, ceftazidime, ciprofloxacin and gentamicin. Isolates resistant to three or all four of mentioned antimicrobials like representatives of different classes of antibiotics with antipseudomonal activity were considered to be MDR.The minimal inhibitory concentrations (MICs) of ceftazidime, ciprofloxacin and amikacin by the E test method (AB Biodisk, Solna, Sweden) were determined according to manufacturer's instructions and evaluated following recommendations of the Clinical Laboratory Standards Institute. Comparison of means between groups was performed by the Student (t) test or Mann-Whitney U test (nonparametric values). Proportions were compared using chi-square or Fisher's exact test. Differences were considered significant at $p<0.05$.

Results

In year 2003, the rate of *P. aeruginosa* strains resistant to piperacillin was predominant (23.3%, n=21), followed by gentamicin (20.0%, n=18) and ciprofloxacin (15.6%, n=14). In year 2008, the prevalence of resistance markedly changed, and currently it was found to be most often expressed to ciprofloxacin (39.6%, n=40) followed by gentamicin (19.8%, n=20) and piperacillin (17.8%, n=18). There was estimated significant increase in resistance to ciprofloxacin (+24%, $p<0.001$) and ceftazidime (+8.3%, $p<0.05$) by years 2003 and 2008. In year 2003, the rate 66.7% (60/90) of strains of *P. aeruginosa* sensitive to all tested antibiotics has significantly decreased as rate 47.5% (48/101) was found in year 2008 ($p<0.05$). In year 2003, the rate 7.8% (7/90) of MDR *P. aeruginosa* has increased insignificantly by 12.9% (11/101) in year 2008 ($p=0.25$). The dominative antibiotic involved in MDR was ciprofloxacin taking part from 85.7% to 90.9% of all MDR isolates in year 2003 and 2008. MICs during periods of study with MIC median 2003 (as baseline) shows significant increase in MIC of ciprofloxacin and amikacin currently as rate of isolates with MIC $\leq$ MIC median 2003 markedly decreased ($p<0.001$), and it was not a case of ceftazidime.

In conclusion, the study showed that overall resistance of *P. aeruginosa* to the antimicrobial agents in the largest University Hospital of Lithuania is relatively high. The resistance to ceftazidime and ciprofloxacin increased more than twofold, while the resistance to other antimicrobials agents was unchanged. MDR resistance also increased almost in half. Ciprofloxacin was the most common component found among MDR isolates.

Keywords *Pseudomonas aeruginosa*, resistance rates

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Survey of *Fusarium* species infecting potato and their resistance to fungicides used in Canada

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Strains of *Fusarium* spp. with resistance to commonly used potato seed-piece treatment fungicides have recently been identified in Canada. In particular, populations of *F. sambucinum* and *F. coeruleum* with resistance to thiabendazole, thiophanate-methyl and/or fludioxonil have been frequently recovered. These pathogen populations have caused an increased incidence of seed-piece decay resulting in poor crop stands and reduced yields. Potato seed tubers with symptoms of decay were sampled from across Canada in spring 2010 to obtain a collection of *Fusarium* isolates. Isolates were identified to species level by microscopic examination of morphological features and molecular methods and then tested for sensitivity to thiabendazole and fludioxonil using a fungicide-amended agar assay. The major seed decay pathogens, *F. sambucinum, F. avenaceum* and *F. coeruleum* were frequently recovered. In addition, isolates of *F. oxysporum* and non-*Fusarium* species such as *Alternaria alternata* and *Rhizoctonia solani* were also identified. Isolates of *F. sambucinum* and *F. coeruleum* varied in their sensitivity to fungicides, with resistance to one or both products found in strains obtained from most Canadian provinces. Isolates of *F. avenaceum* with varying fungicide resistance were also identified, although most isolates of this species were sensitive to both products. Isolates of *F. oxysporum* recovered in the survey were always resistant to fludioxonil, but sensitive to thiabendazole. In summary, the survey confirms the presence of strains of *F. sambucinum* and *F. coeruleum* in Canada resistant to thiabendazole and/or fludioxonil. As well, fungicide resistance in other *Fusarium* spp. was also identified. Fungicide resistance has limited the effectiveness of the most common fungicides used against *Fusarium* spp. on potato seed in Canada. A re-evaluation of management practices for control of *Fusarium*-induced potato seed-piece decay is required.

Keywords: potato; *Fusarium*; fungicide resistance

Tetracline resistant *Escherichia coli* from treated wastewater used for irrigation

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Treated wastewater is commonly used for irrigation in Oman. The aims of the study were to detect antibiotic resistant *Escherichia coli* using disk diffusion method. Fifty seven *E. coli* isolates from treated sewage effluents were tested for susceptibility to sixteen antibiotics. The majority of the isolates exhibited resistance to several antibiotics, most of which were resistant to tetracycline. Plasmid DNA was detected in 84.2% of the isolates, with plasmid number and molecular weight ranging from one to eight and 2.0 kb to 323 kb respectively. Conjugation experiments performed between selected representative strains and recipient *E. coli J53* (sodium azide^{R} (75μg/ml) showed that all transconjugants were resistant to tetracycline and carried a 156.5 kb plasmid DNA. Plasmid curing experiment successfully eliminated all except the 156.5 kb plasmid DNA carrying tetracycline resistance. This strongly suggests that tetracycline resistance is carried on this conjugative plasmid.

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The occurence of Panton-Valentin leucocidin in MRSA strains isolated from hospitalised patients in Slovakia

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The occurrence of Panton Valentin leukocidin in 99 invasive MRSA strains isolated from hospitalized patients was shown to be around 9,1%. Five of nine PVL positive strains had SCCmec type IV casette. The predominating SCCmec type in PVL negative and SCCmec typable MRSA was II. Such strains were simultaneously resistant to erythromycin, clindamycin and ciprofloxacin. The linezolid resistance in invasive MRSA strains from Slovak hospital was not recorded. The MLVA characterization of PVL positive isolates from various locations has demonstrated different patterns. Except one hospital, isolates from over the country expressed the same MVLA pattern.

This study was supported by Pfizer Slovakia grant and by VEGA- 2/0012/08.

Keywords: MRSA, PVL, MLVA, hospital

The role of antimicrobial stress on *Pseudomonas aeruginosa* colony morphology diversity, tolerance and virulence

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In natural environments, as well as in infections, bacteria faced several stresses like starvation, heat exposure, antimicrobials and host defense after entry in human body. The ability to quickly adapt to a new environment is critical to bacteria and the underlying mechanisms are not fully understood. One of the strategies adopted by bacteria is a high frequency of phenotype switching by a mechanism called phase variation. A sign of these bacterial changes is the altered colony morphology on solid media. Several colony morphologies have been isolated from clinical strains, being the best-studied the small colony variants, the rugose small colony variants and the mucoid phenotype.

It was aimed to study the prevalence and diversity of colony morphologies from planktonic and sessile *P. aeruginosa* (Pa) ATCC, chemically stressed, and to compare with the ones developed by a *P. aeruginosa* isolated from a medical device (Pa I). Pa is one of the most important opportunistic pathogen commonly found in clinical arena being often responsible for acute and chronic infections.

Planktonic and sessile Pa and Pa I were *in vitro* stressed by continuous exposure to benzalkonium chloride (BZK) and peroxide hydrogen and by attack with the same products. The stressed bacteria were collected, serial diluted and plated onto TSA to inspect colony morphology variants. Each predominant bacterial morphology was harvested and reserved for further phenotypic and motility characterization.

The results demonstrated that cells coming from biofilm and planktonic growth of Pa, regardless they were stressed or not or the type of stress implemented, develop colonies mostly with the same morphotype, type II, characterized by big and regular colony circumference, with small and dark center and wrinkled surface. This colony type showed to have a good ability to form biofilms, although the colonies from the stressed cultures developed biofilms with higher biomass accumulated.

The Pa I gave rise to high diversity of colony morphotypes, being 3 of them more prevalent and cataloged as type XVII, XXIII, XXVIII. The types XVII and XXVIII are characterized by regular colony circumferences with craters in the center. However their superficial area presented different colors. Type XXIII has irregular colony shape with craters in the center. These 3 morphotypes showed similar biofilm formation ability between them but lower than type II.

Nonetheless the phenotypic differences found between the several morphotypes, all of them generated biofilms with identical tolerance to antimicrobials (BZK and the fluoroquinolone antibiotic ciprofloxacin-CIP). However, the cells resulting from the planktonic growth of Pa I morphotypes demonstrated two-fold tolerance to BZK and CIP than their Pa counterparts.

Regarding bacteria motility, results highlighted that all Pa I morphotypes had impaired swimming motilities compared to type II. This result seems to indicate that the capacity of adhesion or invasion of Pa I morphotypes to, respectively, surfaces or tissues was compromised, which may interfere with their virulence. Although, the latter is not sustained by the susceptibility patterns, emphasizing the ambiguous relationship between virulence and antimicrobial resistance.

The morphologies described are not similar with previous reports and the colony morphologies more prevalent seemed to be less virulent than typical ones. Among the various colony morphologies detected, no Pa I morphotype match with Pa type. So, it can be concluded that phase variation is an adaptive strategy of bacteria to respond to fluctuating environment leading to mixed populations where the chances for survival is higher. The generation of varied bacterial phenotypes may be the sum of previous and successive adaptations suffer by Pa I as an attempt to adjust to adverse habitats.

Keywords: colony morphotypes, biofilm tolerance, antimicrobial stress, virulence, motility

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Transferability of enterococcal *vanA* plasmids among Gram-positive, intestinal bacteria

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Objectives: The most prevalent type of acquired glycopeptide resistance is the *vanA* transposon and its reservoir is in *Enterococcus faecium*. The *vanA* transposon is mainly located on transferable plasmids and why prevalence is mostly limited to *E. faecium* is not known so far. We investigated in vitro transferability of 14 pre-characterized *vanA* containing plasmids (various clinical and non-clinical strains, from different countries, various *repA* classes, etc.) and the prototype multiresistance *E. faecalis* plasmid pRE25 into several enterococcal, lactobacterial, lactococcal, and bifidobacterial recipients to elucidate also a possible reservoir of vanA plasmids in other intestinal colonizers knowing to share a common gene pool with enterococci.

Methods: A filter-mating protocol was harmonized using pre-existing protocols of seven participating partner laboratories. All 15 donor strains were mated with three *E. faecium*, three *E. faecalis*, one *Lactobacillus acidophilus*, one *Lactococcus lactis* and two *Bifidobacterium* recipients using the harmonized protocol. Three filter-matings were performed in parallel and transfer rates were calculated. Selected transconjugants were confirmed by assessing their species and antibiotic susceptibilities. Twelve *E. faecium* transconjugants (from twelve different matings) were daily passaged for four weeks in liquid broth without antibiotics to assess stability of resistance properties in the new host.

Results: In total, 282 enterococcal matings and 73 inter-genus matings were performed and evaluated. *vanA* plasmid transfer into recipients of the same species (*E. faecalis* > *E. faecalis; E. faecium* > *E. faecium*) was frequent and detectable at comparably high rates (10e-3 to 10e-5). In contrast, inter-species transfer was far less frequent than intra-species transfer; the former quite often fairly detectable. All recipients of the same species behaved similarly. Inter-genus transfer could not be demonstrated. Acquired resistance properties remained stable in the new host in the absence of selective pressure.

Conclusions: Intra-species transfer of enterococcal *vanA* plasmids was far more frequent than transfer across species or genus barriers and may thus explain the preferred prevalence of *vanA* containing plasmids among *E. faecium*. A reservoir of *vanA* plasmids in non-enterococcal, intestinal colonizers does not seem to be reasonable.

Keywords vanA, plasmid; Tn1546, Enterococcus faecium, VRE

Transmission of *bla*$_{CTX-M}$ among ESBL-producing *E. coli* strains isolated from infected diabetic foot ulcers

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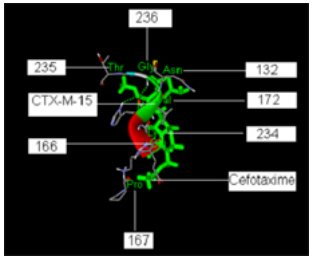
The objectives were to characterize the mode of transmission of *bla*$_{CTX-M}$ and *bla*$_{TEM-1}$ among ESBL-producing *Escherichia coli* strains isolated from infected diabetic foot ulcers, and to identifying the risk factors for "sex-associated multi-drug resistant Gram-negative bacterial (MDRGNB)-infection status" of the ulcers. Seventy-seven diabetic patients having clinically infected foot ulcers were studied in a consecutive series. The *E. coli* strains isolated from the ulcers were screened for *bla*$_{CTX-M}$, *bla*$_{TEM-1}$, *armA*, *rmtA* and *rmtB* during the 2-year study-period. PCR amplified *bla*$_{CTX-M}$ genes were cloned and sequenced. ERIC-PCR was used for the analysis of genetic relatedness of the ESBL-producers. Risk factors for "sex-associated MDRGNB-infection status" of ulcers were assessed. Modeling was performed using Swiss-Model-Server and verified by Procheck and verify3D programmes. Discovery Studio2.0 (Accelrys) was used to prepare Ramachandran plots. Z-scores were calculated using 'WHAT IF'-package. Docking of cefotaxime with modeled CTX-M-15 enzyme was performed using Hex5.1.Among 51 *E. coli* isolates, 14 (27.5%) ESBL-producers were identified. Only 7 Class1 integrons, 2 *bla*$_{CTX-M-15}$, and 1 *bla*$_{TEM-1}$ were detected. Ceftazidime and cefotaxime resistance markers were present on the plasmidic DNA of both the *bla*$_{CTX-M-15}$ positive strains and were transmissible through conjugation. The residues Asn132, Glu166, Pro167, Val172, Lys234 and Thr235 of CTX-M-15 were found to make important contacts with cefotaxime in the docked-complex. Multivariate analysis proved 'Glycemic control at discharge' as the single independent risk factor. This study concludes that male diabetic patients with MDRGNB–infected foot ulcers have poor glycemic control and hence they might have higher mortality rates compared to their female counterparts. Plasmid-mediated conjugal transfer, albeit at a low frequency might be the possible mechanism of transfer of *bla*$_{CTX-M-15}$ resistance marker in the present setting. Amino acids characterized at the binding pocket of enzyme will lead us to design new drug candidate against resistant strains.

Key words: CTX-M, ESBLs, Diabetic foot ulcer



Treatment of infection associated with cochlear implant

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Introduction

Device-related infection with cochlear implantation is difficult to eradicate because bacteria that cause infections live in well-developed biofilms. Clinically that has a major significance as it are resistant to antibiotics, and so causes intractable infections and device extrusions necessitating device removal, with loss of function.

More information is needed about therapy, related to the device infection.

This study aimed to investigate antimicrobial treatment of an infected cochlear implant, undertaken in an attempt to salvage the infected device.

Case report

A 52-year-old man suffering from bilateral sudden deafness since early adult received a Nucleus 24 cochlear implant with the contour advance electrode in his right ear in October 2009. 3 months after the surgery, he felt an increasing retro-auricular pain, and the site of the processor became swollen. The patient was given intravenous ceftriaxone, followed by amoxicillin/clavulanate (orally) for 2 week post-surgery. Thereafter, frequently, he was managed with hospitalization, prolonged courses of antibiotics, and surgical interventions. However, it was resistant to all conventional antimicrobials.

I would like to report a patient who developed severe wound infection after cochlear implantation. This patient was given oral rifampin and TMP-SMX for 4 months and he responded to this treatment, thus preventing explantation and reimplantation.

Conclusion

Ripampin and TMP-SMX may reduce the incidence of revision surgeries/ explantations in severe recurrent infection related cochlear implant infections.

Use of antibiotic resistance genes as marker genes in genetically modified plants

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Presence of antibiotic resistance (AR) genes as markers in genetically modified (GM) plants, such as the *npt*II gene in the GM potato recently authorised for commercialisation in Europe, has raised public concerns about the possibility that these genes would be transferred from GM plants to bacteria and, thus, contribute to the spread of antibiotic resistance in bacteria with potential public health consequences.

The antibiotic resistance traits as present in GM plants are evaluated on a case-by-case basis with respect to their safety for humans, animals and the environment by the Panel on Genetically Modified Organisms (GMO) according to the scientific principles expressed by the Directive 2001/18/EC of the European Parliament and the Council and detailed by the guidance documents of the European Food Safety Authority (EFSA). The evaluation is based on molecular, biochemical, toxicological and environmental evidence.

Following a request from the European Commission to EFSA the GMO Panel and the Panel on Biological Hazards (BIOHAZ) delivered a joint scientific opinion on the use of AR genes as markers in GM plants. This joint opinion focuses on the two AR markers that are present in GM plants for which an application has been submitted to EFSA. One of the AR genes is functional in the plant (*aph(3')*-IIa also known as *npt*II, conferring resistance to kanamycin/neomycin); the other AR gene (*ant-(3'')*-Ia also known as *aad*A; conferring resistance to streptomycin/spectinomycin) is not expressed in the GM plants as the expression is regulated by a bacterial promoter not active in plants.

From all the evidence gathered, the two Panels drew, among others, the following conclusions:

The transfer of AR marker genes from GM plants to bacteria has not been shown to occur either in natural conditions or in the laboratory in the absence of sequence identity in the recipient bacterial cell. Sequence identity is necessary to allow homologous recombination between the transformed DNA in the plant and bacterial DNA.

Recent metagenomic analyses of total bacterial populations (including non-cultivable bacteria) have demonstrated that resistance determinants of kanamycin, neomycin and streptomycin are present in all environments investigated. Such resistance genes may be selected from this environmental reservoir and disseminated among bacteria.

The antibiotic resistance marker genes, *aph(3')*-IIa (*npt*II) and *ant(3'')*-Ia (*aad*A), in GM plants are of bacterial origin. These AR genes occur at different frequencies in different species, isolates and different environments, in naturally occurring bacteria.

There are limitations related among others to sampling, detection, challenges in estimating exposure levels and the inability to assign transferable resistance genes to a defined source. The importance of taking these and other uncertainties into account requires to be stressed.

Notwithstanding these uncertainties, the current state of knowledge indicates that adverse effects on human health and the environment resulting from the transfer of these two AR genes from GM plants to bacteria, associated with use of GM plants, are unlikely.

Two members of the BIOHAZ Panel expressed an alternative view regarding the last conclusion in minority opinions.

Keywords antibiotic resistance marker; horizontal gene transfer.

* EFSA GMO Panel Members (2006-2009): Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Patrick du Jardin, Niels Bohse Hendriksen, Lieve Herman, Sirpa Kärenlampi, Jozsef Kiss, Gijs Kleter, Ilona Kryspin-Sørensen, Harry A. Kuiper, Ingolf Nes, Nickolas Panopoulos, Joe Perry, Annette Pöting, Joachim Schiemann, Willem Seinen, Jeremy B. Sweet and Jean-Michel Wal.

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† EFSA BIOHAZ Panel Members (2006-2009): Olivier Andreoletti, Herbert Budka, Sava Buncic, Pierre Colin, John D Collins, Aline De Koeijer, John Griffin, Arie Havelaar, James Hope, Günter Klein, Hilde Kruse, Simone Magnino, Antonio Martínez López, James McLauchlin, Christophe Nguyen-The, Karsten Noeckler, Miguel Prieto Maradona, Birgit Noerrung, Terence Roberts, Emmanuel Vanopdenbosch, Ivar Vågsholm

Vancomycin resistant enterococci (VRE) in equine-faecal samples

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The occurrence and prevalence of vancomycin resistant enterococci (VRE) in equine-faecal samples of hospitalized (n=66) and non-hospitalized (n=72) horses were investigated in North West region in England in order to evaluate any potential role of horses to harbour this zoonotic pathogen. Modified culture methods with presumptive selection were used for *Enterococcus spp*. and VRE. A multiplex polymerase-chain reaction (PCR) method was used for genotyping of VRE. Overall 47 identified VRE isolates (from 264 faecal samples from 138 horses) were collected and only 9 isolates were confirmed and characterized by PCR. The *VanC* genotype (i.e. *vanC-1* gene) was identified in seven isolates and of these, six were from hospitalized horses. One positive isolate of each of *VanA* and *VanD* genotype were also detected both in non-hospitalized horses but were untypable by PCR. Furthermore, susceptibility testing by disc diffusion showed that all isolates (n=9) exhibited further resistance to other antibiotic classes. Unlike *VanA* genotype, which is reportedly associated with human infections, PCR revealed that genotypes of most isolates (i.e. VanC genotype) were not common to clinical human isolates. This study suggests that horses are unlikely to have a major role in the zoonotic transmission of VRE in this geographic area. Furthermore, there is an increased apparent prevalence of VRE in hospitalized horses suggesting a possible nosocomial transmission in hospital.

Keywords: Equine; Vancomycin resistant enterococci; Nosocomial infection; *VanC* genotype; *Enterococcus gallinarum*

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Vancomycin tolerance in *Streptococcus pneumoniae* depends on reduced enzyme activity of the major LytA autolysin or cooperation between CiaH histidine kinase and capsular polysaccharide

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Streptococcus pneumoniae is the leading cause of pneumonia and meningitis in children and older adults. Despite advances in medical care, current estimates of mortality due to pneumococcal meningitis ranges from 16% to 37%. The worldwide emergence of β-lactam-resistant strains of *S. pneumoniae* has complicated the antimicrobial treatment of invasive pneumococcal diseases particularly meningitis. Given reports of frequent meningitis treatment failures as a result of multidrug-resistant bacteria, vancomycin is often added to standard therapy regimens for meningitis. Although vancomycin-resistant *Streptococcus pneumoniae* strains have not been isolated, reports on the emergence of vancomycin-tolerant pneumococci are a cause of concern. Tolerance is defined as a change in the activity of an antibiotic that affects cell wall synthesis from bactericidal to bacteriostatic. To date, the molecular basis of vancomycin tolerance in *S. pneumoniae* is essentially unknown.

We examined two vancomycin-tolerant clinical isolates, i.e. a purported autolysin negative (LytA$^-$), serotype 23F isolate (strain S3) and the serotype 14 strain 'Tupelo', which is considered a paradigm of vancomycin tolerance. S3 was characterized here as carrying a frameshift mutation in the *lytA* gene encoding the main pneumococcal autolysin. The vancomycin tolerance of strain S3 was abolished by transformation to the autolysin-proficient phenotype. The original Tupelo strain was discovered to be a mixture: a strain showing a vancomycin-tolerant phenotype (Tupelo_VT) and a vancomycin-nontolerant strain (Tupelo_VNT). The two strains differed only in terms of a single mutation in the *ciaH* gene present in the VT strain. Most interestingly, although the vancomycin tolerance of Tupelo_VT could be overcome by increasing the LytA dosage upon transformation by a multicopy plasmid or by externally adding the autolysin, we show that vancomycin tolerance in *S. pneumoniae* requires the simultaneuous presence of a mutated CiaH histidine kinase and capsular polysaccharide.

Keywords *Streptococcus pneumoniae*; antibiotic tolerance; vancomycin; two-component system; CiaH

Viewing evolution and global spread of drug resistant bacterial pathogens through whole genome sequencing

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Current methods for differentiating strains of pathogenic bacteria provide low granularity data with limited resolving power so are unable detect base-specific evolutionary events and precise relationships between isolates. Using second generation genome sequencing technology, we can precisely differentiate isolates providing a high-resolution view of pathogen epidemiology. Applied to globally dominant lineages of Staphylococcus aureus and Streptococcus pneumoniae, this approach reveals global geographic structure within the lineage, evidence of intercontinental transmission, acquisition of antibiotic resistance, vaccine evasion and potential for detection of person-to-person transmission within a hospital environment. These results demonstrate a major technological advance in our ability to interrogate and resolve bacterial populations that will have a dramatic effect on future strategies for control, treatment and prevention of infectious disease.

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White swine as a re-emerging reservoir of antimicrobial-resistant nontyphoidal *Salmonella*

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Salmonellosis is the most important food-borne disease in the North Hemisphere. Although *S.* Enteritidis (from poultry sources) is the main responsible for human infections, *S.* Typhimurium (from pork products mainly) has been increasing its prevalence in recent years (EFSA, 2009). The potential transmission of antimicrobial resistance microorganisms from livestock animals to humans is well known but not all production systems are equally responsible (Aarestrup, 2006). In the case of swine-farming in Spain, a sharp contrast there exists between white and Iberian pigs, being the later associated to more extensive breeding management and, presumably, to a lower exposure to antimicrobial treatments.

The collection of strains analyzed in this work includes multi-resistant salmonellas isolated from white swine (WS; n=39) or Iberian swine (IS; n=94) at slaughterhouses (the University of Cordoba and the VISAVET Health Surveillance Centre at the University Complutense of Madrid). In order to realize comparative studies with bacteria isolated from humans, 303 salmonella strains were isolated at the six hospitals of the Extremadura Health System. Interestingly, the number of *S.* Typhimurium isolates from humans was slightly higher than that of *S.* Enteritidis (113 over 102). The analysis of clonal relationships by PFGE revealed that three pulsetypes were the most prevalent (n>10), which are: *S.* Enteritidis EN02 (n=71), *S.* Typhimurium TY09 (18 from human clinical cases and 12 from WS) and *S.* Typhimurium TY19 (12 human strains).

Minimum inhibitory concentrations (MIC's) have been calculated for 23 antimicrobial agents whose monitoring has been recommended by the EFSA (European Food Safety Agency). Almost all *S.* Typhimurium strains presented multi-resistant phenotypes, with little or no differences between WS, IS or human isolates. Among the genetic elements that carry antimicrobial resistance genes and that are involved in their horizontal transference, class 1 integrons have a remarkably important role, and thus, the resistance genes contained in gene-cassettes associated to class 1 integrase genes (*int*1) were screened by PCR on the *Salmonella* strains. These genetic elements were particularly abundant among *S.* Typhimurium from animals, mostly from WS, which presented a 47% of positive strains, in contrast to human isolates, where only one fifth were positives. Among twelve different RFLP profiles in which gene-cassettes were classified, only two were shared among *S.* Typhimurium from WS, IS and humans. The first was found associated mainly to the *S.* Typhimurium TY09 clone (only one strain from IS), whilst the second was detected in six different serovars (Brandenburg, Bredeney, Hindmarsh, Anatum, Choleraesuis and Typhimurium), facts that evidences their mobilization by horizontal transference. Finally, the high prevalence of *S.* Typhimurium might indicate an increasingly importance of pork meat as source of salmonella infections in humans. This work evidences the potential risk for spreading the antimicrobial multi-resistance and points out the importance of considering animal production and management programmes when food chain surveillance is implemented.

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EFSA, 2009, Trends and sources of zoonoses and zoonotic agents in the European Union in 2007, Parma.

Keywords: *Salmonella* Typhimurium; multi-resistance: class 1 integron; swine; human; Spain

8. Chemistry

"Trojan Horse"'s solution: an efficient strategy against multidrug resistance bacteria

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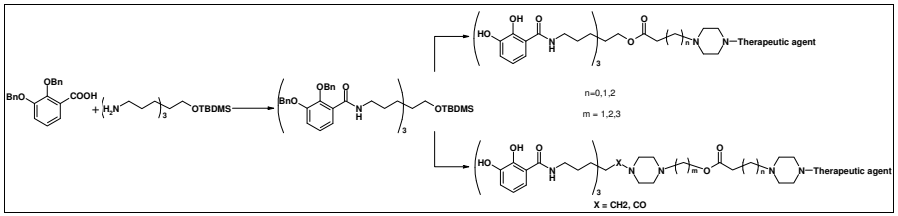
Multi-drug resistant bacteria (MDRB) are a burning issue in our healthcare system. In nowadays about 10 % of patient hospitalizations in industrious countries are due to a nosocomial infection. However pharmaceutics firms and laboratories have not provided new drugs in time to treat bacteria that became resistant to older antibiotics. These MDRB have created a great urge to propose new chemical antibacterial agents.

Two important bacterial resistance mechanisms are notably involved with the MDRB phenomenon: firstly, a decreased permeability of the bacterial membranes towards some antibiotic drugs, secondly, a low intra-microbial concentration of antibiotic due to the bacterial efflux system. On the same time bacteria synthesise, for their development, molecules called siderophores which bind extracellular ferric iron in order to transport it into their cells. It has been reported that molecules with an iron (III) chelator part, which mimics a siderophore, linked with an antibiotic agent enhance the action of this latter, following a "Trojan Horse" strategy. Furthermore the inhibition of the bacterial efflux system has been shown to increase the therapeutic effect of antibiotic on MDRB.

In order to develop new therapeutic agents against multi-drug resistant bacteria, we decided to work on siderophore-linked antibiotics: an iron(III) chelator linked by a cleavable chemical bond to a therapeutic agent. We reported here, the first part of our work, concerning the synthesis and preliminary biological results of the catecholamides attached to a functionalized multi-amine backbone with a cleavable bond (scheme 1) to a therapeutic agent (like an antibiotic (βlactam, fluoroquinolone, oxazolidinone...) or an efflux system inhibitor).



Scheme 1

Keywords resistance, efflux pump, iron chelation, "Trojan Horse"'s strategy, antibiotic

Amphiphilic dendrimeric peptides with affinity to microbial membranes - polyvalency vs. selectivity

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Dendrimers are synthetic macromolecules of nanoscopic dimensions built from several layers of branches assembled around a central core,. They are characterized by a high number of functional groups at the surface. Unlike other macromolecular compounds, their unambiguous composition, reliability and versatility of their synthesis, make this type of molecules well-suited to various medical and biochemical applications. Previously designed in our group low molecular weight peptide dendrimers built from basic amino acids (Lys, Orn, etc) exhibited good activity against *S. aureus* and moderate against *E. coli* and *C. albicans*. As evidenced by electron microscopy, model DSC studies and similar potency of dendrimers containing D- and L- enantiomers, they preferentially interact with microbial membranes [1-4].

Here we present divergent synthesis, characterization and activity against *S. aureus, E. coli,*and *C. albicans* strains of a two libraries of amphiphilic peptide dendrimers that have different topology and number of positively charged and lipophylic groups. In particular, functionalization of C-end of a core fragment of these dendrimers by various lipophylic and polar groups enhanced their potency and directed their selectivity towards *Candida* genus along with decrease of toxicity (improved therapeutic index). It has been found by CD spectroscopy, that enhanced potency of the designed dendrimers correlate with higher content of β-structure, i.e. ability to form aggregates in solution.

Key words: antimicrobial, dendrimers, *S. aureus, E. coli, C. albicans*, selecticvity, toxicity

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Antibacterial Activities of Synthetic Compounds Containing Copper(II) and Guanidine Derivatives.

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Two copper(II) complexes, $[Cu(L^1)_2]Cl_2$ (**1**) and $[Cu(L^2)_2]Cl_2$ (**2**) (L^1 = amidino-*O*-methylurea; and L^2 = *N*-methylphenyl-amidino-*O*-methylurea), were synthesized by methanolysis of cyanoguanidine (for **1**) or (*N*-methylphenyl)-cyanoguanidine (for **2**) in the presence of copper(II) chloride. The obtained products were then characterized by CHN elemental analysis and spectroscopic methods (infrared, diffuse reflectance and mass spectrometry). Investigation on the antibacterial activities of **1** and **2** toward *Campylobacter* has revealed inhibition zones of 9.0 and 14.5 mm, repsectively, corresponding to the minimum inhibitory concentration (MIC) values of 1.56 and 0.78 mg mL^{-1}, respectively. The considerable better reactivity of **2** may result from the different structural feature on the ligands in which the aromatic moieties on the *N*-substituted sidearm groups of the L^2 ligand could enhance the activity.

Keywords: Copper(II); Guanidine derivatives; Antibacterial; *Campylobacter*

Antimicobacterial dispersions from tuberculostatics drugs and dioctadecyldimethylammonium bromide (DODAB) bilayers

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Cationic bilayers in form of bilayer fragments (BF) or large vesicles (LV) provide adequate environment for solubilization and stabilization of antimicrobial drugs with the advantage of being also antimicrobial agents. In this work, BF or LV interaction with two tuberculostatic drugs, rifampicin (RIF) and isoniazide (ISO) is characterized. RIF and ISO present low and high solubility in water, respectively, so that ISO was expected to be entrapped in the vesicle water compartment whereas RIF was expected to adsorb and/or insert in the lipid bilayer. Methods were determination of entrapment efficiency for both drugs from dialysis experiments and determination of particle size by dynamic light scattering. The interaction between bacteria and DODAB bilayer was evaluated from cell viability, zeta potential measurements and DODAB adsorption isotherms onto cells. LV were leaky to ISO whereas RIF could be incorporated in the cationic bilayer at high percentiles. RIF drug particles above its solubilization limit could be solubilized by BF at 0.5 mM lipid. The cationic lipid alone killed *Mycobacterium* over a range of low concentrations. The mechanism of action of DODAB is related to change of charge of the bacterial cell when cell charge changes from negative to positive. Through the adsorption isotherms it was possible to conclude that DODAB adsorbs onto mycobacteria with high affinity during the interaction. The novel assemblies may become useful in chemotherapy against tuberculosis.

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Antimicrobial activity ligands and their corresponding palladium(II) complexes against *Aspergillus* species

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For eleven ligands alkyl esters (ethyl, propyl, butyl and pentyl) of some tetradentate ligands of eddp-type ((*S,S*)-ethylenediamine-*N,N`*-di-2-propanoate, (*S,S*)-ethylenediamine-*N,N`*-di-2-(3-methyl) butanoate, (*S,S*)-ethylenediamine-*N,N`*-di-2-(4-methyl) pentanoate) and corresponding palladium(II) complexes antimicrobial activity is investigated. Testing is preformed by microdilution method and minimum inhibitory concentration (MIC) and minimum microbiocidal concentration (MMC) have been determined. Testing is conducted against *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and *Aspergillus restrictus*.

Minimum inhibitory concentration with ligands ranged from 39.06 μg/mL to >5000 μg/mL, minimum microbiocidal concentration from 78.125 μg/mL to >5000 μg/mL. With palladium(II) complexes minimum inhibitory concentration ranged from 7.8125 μg/mL to 5000 μg/mL, minimum microbiocidal concentration from 31.25 μg/mL to 5000 μg/mL. Tested ligands, with few exceptions, show very low antimicrobial activity. Palladium(II) complexes show selective and moderate activity. The difference in antimicrobial activity between ligands and corresponding palladium(II) complexes is noticed and it is, as a rule, always higer with palladium(II) complexes.

Keywords antimicrobial activity; ligands; palladium(II) complexes; *Aspergillus* spp.;

Antimicrobial cyclopeptides including aza-β^3-amino acids

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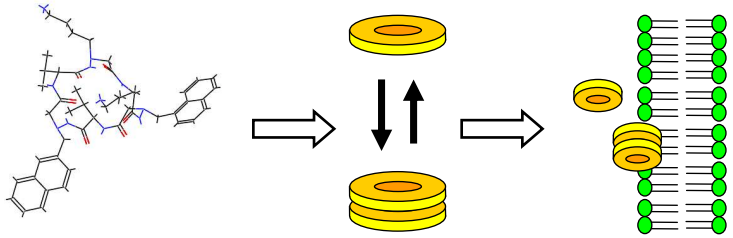
Antibiotic resistance of pathogens against conventional antibiotics is increasing at a rate that far exceeds the pace of new development of drugs. So, antimicrobial peptides, both synthetic and from natural sources, have raised interest as potential useful drugs in the future.

However, due to proteolytic degradation, peptides are not ideal candidates for pharmaceutical development. That why numerous research try to develop non natural peptidic analogues for enhance metabolic stability, bioavailability, and biological absorption. In this class of peptidomimetics, pseudopeptides consisting exclusively or including aza β^3-amino acids have emerged as a promising new class of compounds that favour hydrogen bond formation and can enhance biological activities of natural parent peptides. [1]

We have designed "mixed" cylic pseudopeptides composed of α- and aza-β^3-amino acids that targets bacterial cell wall and induce the death of the pathogens. Particularly, some of these cyclic pseudopeptides have broad spectrum antibactericidal activities on gram positive and gram negative bacteria with low minimum inhibitory concentrations (MIC). On the other hand, this type of molecules is not haemolytic and cytotoxic at antimicrobial activity levels. [2].



We try to explain the mechanism of action of our pseudopeptides that act on the microbial membranes. With NMR studies we have demonstrate that in solution cycles auto-associate at high concentrations and we investigate their compartments with lipids in presence of small unilamellar vesicules (SUV).[3] This phenomenon of auto association due to aromatic stacking is facilitated at the surface of the microbial membranes and is responsible of biological activities.

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Keywords pseudopeptides; auto-assembling, antimicrobial

Bacterial toxin-antitoxin interaction as a new target for antimicrobials

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Bacterial toxin-antitoxin (TA) systems were originally discovered in plasmids but now their presence is confirmed in other mobile genetic elements and in most of the chromosomes of Eubacteria and Archaea [1].

Most of the type II TA systems have a similar genetic organization: an unstable antitoxin followed by a stable toxin. Toxins are proteins which inhibit cellular growth or viability if their antitoxins are absent. The antitoxin is a protein able to neutralise the bacteriostatic or bactericidal effect of the toxin. In contrast to the toxins, the antitoxins are less stable as they are more sensitive to cellular proteases [2].

This type of systems have been pointed as new targets for antimicrobial as molecules able to inhibit the neutralising toxin-antitoxin interactions would leave the toxin free to inhibit cell growth and viability. TA interactions have to be detected in order to perform high-throughput screenings for searching molecules with inhibitory potential. There are at least two techniques useful to detect protein-protein interactions that are amenable to this type of screenings: BRET (Bioluminescence Resonance Energy Transfer) and FRET (Fluorescence Resonance Energy Transfer) [3]. Both are based in resonance energy transfer between two molecules. In BRET, transfer occurs between a bioluminescent protein and a fluorescent one, while in FRET energy transfer occurs between two fluorescent proteins. Both techniques can be used both *in vitro* and *in vivo* assays to quantify the interaction between the proteins. Up to now, BRET has been used to evaluate TA interactions in two systems of Gram-positive bacteria, *relBE2* of *Streptococcus pneumonie* [4] and *ω-ε-ζ* of *Streptococcus pyogenes* of pSM19035 plasmid [5].

We now extend this analysis to one of the best characterized TA systems of enterobacteria, the *parD (kis, kid)* system of the antibiotic resistance factor R1 of enterobacteria. The toxin of this system, the Kid protein, is a specific endo-ribonuclease that inhibits protein synthesis and interferes with cell growth and viability. Interaction with the Kis antitoxin neutralizes the RNase activity of the toxin [6, 7]. *parD* has homologues in the *Escherichia coli* chromosome and also in the chromosomes of other main pathogenic bacteria such as *Mycobacterium tuberculosis* or *Staphylococcus aureus*. We have performed BRET and FRET assays to follow Kid-Kis interactions. For BRET assays we have selected *Renilla reniformis* luciferase protein (Rluc) and Enhanced Yellow Fluorescent Protein (EYFP) whereas for FRET assays monomeric Cyan Fluorescent Protein (mCFP) and EYFP have been used. In both cases Kid toxin N-terminal end was fused to EYFP, whereas Kis antitoxin was fused either to the C-terminal end of the bioluminescent or fluorescent protein. The comparative analysis of TA interactions based in both assays will be presented. We aim to develop a protocol that could be adapted to high-throughput screening of molecules able to distort the TA interactions activating the toxic activity of Kid. The detailed structural and functional knowledge available on the Kis and Kid proteins and their interactions [7] sets up an optimal starting point for these developments.

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Keywords toxin-antitoxin systems; protein-protein interactions

Benzoquinones secreted by Tribolium beetles found to be selective antimicrobials

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Ever since the late 1800's, it has been known that Tribolium beetles, a common pest of stored grain, secreted odiferous chemicals. In the mid-twentieth century these compounds were identified as derivatives of benzoquinones and theories as to their function abounded. Most of these hypothetical functions revolved around protection from perceived threats to their survival including rats, parasites, con-specifics, and microbes. Although some of these hypotheses were tested throughout the later half of the last century, no final determination was made. A concerted effort with my undergraduate students has meticulously tested the role of these compounds. As a result we have determined that the compounds are not an effective deterrent of rats but do control microbial growth in their flour environment at the levels produced by the beeltes. This anti-microbial effect is not universal. When tested against type specimens known to be found in flour, the compounds where found to be much more effective against bacterial species than yeast species. This result makes evolutionary sense since yeast species are the main carbon source for the beetles, whereas several of the bacterial species have been shown to cause harm to the beetles. Additionally, integration of the outcomes from other experiments with our results suggests an interesting relationship between benzoquinone production and infection of the beetles with the rat tapeworm *Hymenolepis diminuta.* In order to complete the understanding of how these chemicals serve these beetles, we have also completed genetic analyses (QTL studies) that have identified almost two-dozen loci that contribute to this trait. We are currently in the process of using this information to determine the metabolic pathway responsible for benzoquinone production as well as to better define the epigenetics that control these anti-biotic secretions.

Keywords benzoquinones, Tribolium

International Conference on Antimicrobial Research (ICAR2010)
Valladolid (Spain), 3-5 November 2010

Characterization of DnaG as a potential target for new drugs against tuberculosis

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Tuberculosis (TB) is an important infectious disease, causing high morbidity and mortality worldwide. The situation is made even worse by the emergence of drug-resistant *Mycobacterium tuberculosis* strains. The list of drugs effective in TB treatment is short, especially that the effective therapy is based on a cocktail of at least three drugs. It is generally accepted that new efficient anti-TB drugs and alternative drug targets are required to control drug-resistant forms of TB. Such targets should be essential for bacteria and not present in the human host. DnaG has been proposed to be one of such candidates.

Primase DnaG plays the key role in a DNA replication mechanism. It is the ssDNA-dependent RNA polymerase, that synthesizes short RNA primers to initiate DNA replication. DNA primases are divided into two groups. The first one consist of bacterial and bacteriophage enzymes, such as DnaG, while the other one includes the major heterodimeric eukaryotic primases, that form a complex with DNA polymerase alpha and its accessory B subunit. The proteins of these two classes differ both in structure and in their spatial relationship with other proteins in the replication complex. Therefore bacterial primase is an attractive target for therapeutic intervention. In this work, we undertake a series of experiments that permit the analysis of indispensability of *dnaG* in mycobacteria and examine the possibility of replacing this gene by other primases in conditions of their overproduction. The homologous recombination was used to replace *dnaG* with its unfunctional (ΔdnaG) copy.

The essentiality of *dnaG* was evaluated in wild type *M. smegmatis* strain and in engineered mutants with a primase overproduction background. The wild type intact gene was replaced with unfunctional copy only when *M. tuberculosis* or *M. smegmatis dnaG* was expressed from an extra copy controlled by inducible promoter, but not in *E. coli dnaG* or *M. smegmatis* "eukaryotic like" primases (Prim 1, Prim 2, Prim 3) overproduction background.

We conclude that DnaG is essential for viability of mycobacteria and it is a potential useful target for a new anti-tubercular drugs.

Keywords DnaG, Mycobacterium,

Chemical modification of macrolide antibiotics of oligomycin and amphothericin groups; SAR of derivatives

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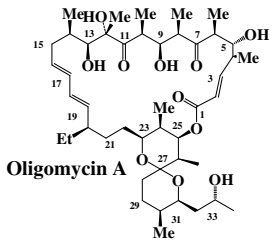
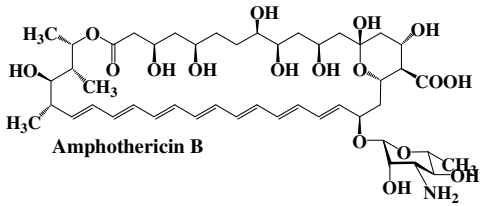
The first examples of chemical modification of antibiotic oligomycin A are presented. Annulated heterocyclic structures are formed after the interaction of the antibiotic with hydroxylamine, N-aminopyridine or NBS. Retrolaldol degradation of the antibiotic leading to a novel macrocycle structure is shown. Chemical modification of novel polyene macrolides of Amphothericin A type obtained by genetic engineering methods (prof. S. B. Zotchev. Norway) in the polyol region and exocyclic carboxyl group led to the derivatives with lower toxicity and higher antifungal activities *in vivo*. The role of modification of various structural moieties of these antibiotics is discussed.



Oligomycin A



Amphothericin B

Keywords oligomycin A, amphothericin type antibiotics, chemical transformation

Comparative study of binary and ternary copper complexes of ciprofloxacin

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Quinolones are amongst the most widely prescribed families of antibiotics, both in human and veterinary medicine, due to their broad spectrum of activity and safety profile [1]. However, their overuse/misuse seems to be the basis of the emergence and dissemination of microbial resistance that results from the bacterial adaptations and compromises antimicrobial efficiency [2].

Ciprofloxacin is a second generation quinolone which gain notority back in 2001 due to its efficacy agains the anthrax (*B. anthracis*) agent. The quick development of resistance to antibiotics by microorganisms has lead to the need for constant reinvention of these drugs, a context in which the metalloantibiotics can arise as a possible solution [3].

Solution behaviour of ciprofloxacin complexes with copper(II), nickel(II), cobalt(II) and zinc(II) in the presence and absence of 1, 10-phenanthroline was studied in aqueous solution, by potentiometry. The results obtained show that under physiological conditions (micromolar concentration range and pH 7.4) only copper(II) forms stable complexes. Binary copper(II)/ciprofloxacin and ternary copper(II)/ciprofloxacin/phenanthroline complexes were synthesised and characterized by elemental analysis, UV-visible spectroscopy and FTIR. The antimicrobial activity of these complexes and of copper(II)/ciprofloxacin and copper(II)/ciprofloxacin/phenanthroline solutions, prepared by mixing of the individual components in the same stoichiometric proportion and concentration range used for the synthesised complexes, were tested against two different *E.coli* strains. Although, at a glance, the results point to a possible use of both complexes as metalloantibiotics, a detailed analysis shows that, at biological concentrations, the copper(II) binary complex does not exist and the antimicrobial activity observed is a consequence of its dissociation into free ciprofloxacin. Consequently, only the ternary complex seems worth pursuing as a possible antimicrobial agent candidate.

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Keywords Ciprofloxacin, microbial susceptibility, metallocomplex

Delivery of anti-listerial peptides from plant seeds by artificial digestion system TIM1

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The TNO artificial GI system could be a useful tool in pharma-related studies for following the intralumenal fate of a drug compound or a probiotic strain under dynamic conditions. For instance, TIM-1 could be used to determine where and when a compound is released, what might influence its release, its stability or viability and its availability for absorption, and what role the presence of food, transit time, or drug delivery systems could play in these processes. The gastric small-intestinal model represents the stomach, duodenum, jejunum and ileum. The pH curves, peristaltic movements, gastric emptying, intestinal transit and gradual additions of digestive juices are computer-controlled events, comparable to human conditions. We have previously demonstrated the presence of antimicrobial peptides (AMPs) in some extracts issue from plant seeds. The present study was aimed at investigating the antibacterial activity of different fractions resulting from the TIM-1 digestion of an antimicrobial peptidic extract from the plant *Oudneya africana*. Futhermore, the generability of AMPs from inactive plant extracts using the artificial digestion system TIM-1 was investigated. The resulting extracts were physico-chemically characterized.

Keywords plant antimicrobial peptides; Gastric-small intestinal system TIM-1; peptide release

International Conference on Antimicrobial Research (ICAR2010)
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Effect of Agar and Arabic Gum on the Kinetics of Inactivation of Lysozyme Stored at Different Temperatures

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Lysozyme is an enzyme with antimicrobial activity witch is abundantly found in biological sources. It's application as a natural antimicrobial agent in foods is being thoroughly investigated. The purpose of this search was to investigate the effect of hydrocolloids agar and Gum Arabic on the lysozyme activity stored at different temperatures. Solutions of hen egg white lysozyme (HEL) containing 1% agarose or 20% Gum Arabic in phosphate buffer was stored at different temperatures (-18˚, 4˚C, 21˚C and 45˚C). Samples were removed at different time intervals and lytic activity against cell wall of *Micrococcus lysodecticus* was determined. Result showed that inactivation of lysozyme follows a first order kinetics at all temperatures. Presence of agar and Gum Arabic decreased the initial of activity of lysozyme by 50% and 75%, respectively. However, no significant difference was observed in the first order rate constants at different temperatures in the presence and absence of hydrocolloids. Arrhenius plots the activation energies (Ea) was 38.7 - 42.6 Kcal/mole at 21- 45˚C and 0.34-1.7 Kcal/mole at -18 – 21˚C, indicating changes in the temperature sensitivity of the inactivation when lysozyme is stored at high and low temperatures. The results of this study indicates lyzozyme might lose most of its antimicrobial activity in foods stored at high temperature and can be used in food stored at low temperatures and presence of hydrocolloids decreases lysozyme a activity at all temperatures. Under theses conditions one might need to use higher concentrations of lysozyme to achieve adequate antimicrobial effect.

Key words: lysozyme activity, inactivation, kinetics, , agar, gum Arabic

Effect of Paracetamol on the Pharmacokinetics of Cephalexin in Dogs

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The pharmacokinetics of single oral dose (20 mg kg^{-1} b.wt.) of cephalexin alone and concomitantly with paracetamol was studied in normal healthy dogs. Moreover, the effect of simultaneous co-administration during five continuous days of treatment on hepato-renal functions was also evaluated. Cephalexin was rapidly absorbed from the gastrointestinal tract and the calculated peak serum concentration of cephalexin C_{max} was 16.47 µg ml^{-1} attained at 1.96 h (t_{max}). Paracetamol treatment significantly decreased the cephalexin concentrations from 30 minutes till 4 hours post administration. Paracetamol significantly lowered the peak serum concentration and the area under the concentration curve AUC of cephalexin. The relative bioavailability (F_r) of cephalexin with paracetamol was 76.65%. The percents of cephalexin protein binding in normal dog's serum either alone or in combination with paracetamol were 15.60, and 14.24 % respectively, indicating that the co-administration were significantly decreased the binding tendency. Following multiple doses of cephalexin alone, AP, ALT and AST activities, urea and creatinine were significantly increased till the fifth day then returning to the normal level. While concomitant administration of cephalexin with paracetamol induced transient significantly increases in the concentrations of urea and creatinine during the treatment period. Therefore, concomitant use of paracetamol with cephalexin may require close monitoring for clinical consequence of potential drug interaction.

How NMR diffusion and ^{13}C relaxation measurements in solution can provide insight into the pore formation mechanism of a cyclic lipodepsipeptide

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Pseudodesmin A[1] is a cyclic lipodepsipeptide (CLP) that belongs to the viscosin group, a series of CLPs produced by *Pseudomonas* bacteria which often possess potent antimicrobial activity. It consists of a nonapeptide chain, containing both L- and D-amino acids, with the N-terminus bound to a fatty acid chain and the C-terminus involved in a lacton (depsi) bond with an alcohol group of a Thr residue in the middle of the chain, effectively forming the cyclic structure. For this group of compounds, it has been assumed that the biological activity is caused by the capacity to form ion pores in cellular membranes. Pore formation requires that these small molecules self-assemble into larger structures within the non-polar membrane environment so as to be capable to traverse the bilayer. With solution NMR spectroscopy, we have found in non-polar organic solvents such as chloroform that pseudodesmin A forms large supramolecular structures which can be linked to the ion-pore forming capability[2]. Using translational diffusion NMR measurements, we show that the self-assembly is indefinite and that at high concentrations the hydrodynamic radius increases over a factor of 4 compared to the monomer state. The solution structure of an individual pseudodesmin A unit was determined and found to be a small amphipathic left handed α-helix with its C-terminal end covalently connected with the middle of the helix by a three residue loop (Figure 1). Based on these results, a model for the self-assembly was proposed[2], which involves stacking interactions between the complementary ends of the helices, while the hydrophilic sides of the molecules aggregate to minimize the contact between the polar molecular surface and the non-polar environment. This effectively creates a hydrophilic tunnel capable of spanning the membrane[2] (Figure 2).

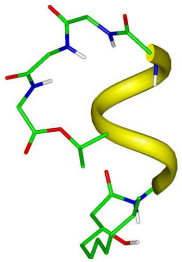

Figure 1

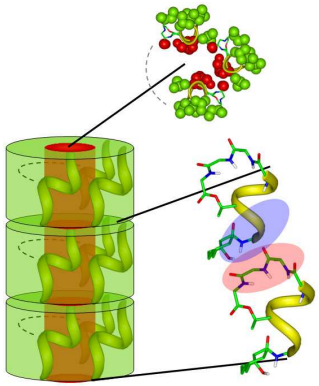

Figure 2

To validate our proposed model, a novel approach was used, consisting of heteronuclear $^{13}C^{\alpha}$ relaxation (T_1 and T_2) NMR measurements performed at different concentrations under self-assembling conditions. In contrast with the translational diffusion coefficient, the NMR relaxation rate constants can be used to obtain the degree of anisotropy and the direction of the anisotropy of a molecular structure. It was found that the self-assembled structures increase in size along only one dimension with increasing peptide concentration (*cf.* a cylindrical shape increasing in length only) and that the direction of growth is parallel to the monomer helix axis, confirming our proposed model.

Our approach provides a new method to characterize the structure of ion pores formed by small self-assembling peptides by directly studying them in organic solvents, provided solvent conditions are identified that promote self-assembly.

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[2] Sinnaeve et al, Chem Eur J 15(2009) 12653-12662, DOI: 10.1002/chem.200901885

Keywords pseudodesmin; ion pore; NMR spectroscopy

Importance of the C9 absolute configuration for the antifungal activity of natural and semisynthetic sesquiterpenes.

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The genus *Polygonum* (Polygonaceae) is represented in Argentina by 21 species which are divided into five sections: *Amblygonum*, *Echinocaulon*, *Tiniaria*, *Polygonum* and *Persicaria*. Most of these species are reported to have interesting biological activities.[1] In a previous work we reported the isolation of two sesquiterpene dialdehydes [polygodial (**1**) and isopolygodial (**2**)] and a related alcohol [drimenol (**3**)] from *Polygonum acuminatum* Kunth, a species belonging to *Persicaria* section.[2] In this abstract we describe the obtainment of isodrimenol (**4**) by semisynthetic means, and the evaluation of the antifungal activities of the four compounds.

Compounds **1**-**3** were isolated from dichloromethane extracts of leaves of *P. acuminatum* collected in summer 2008 and compound **4** was prepared by catalytic hydrogenolysis of isodrimendiol (**5**) which, in turn, was obtained by reduction of **2** with sodium borohydride (1:4). Figure 1.

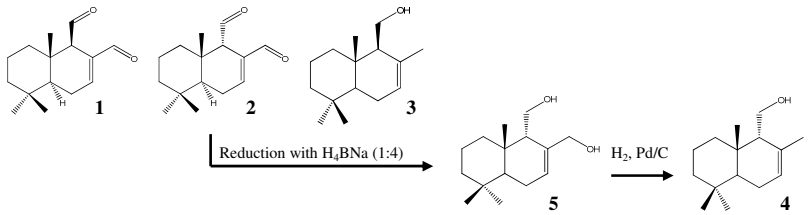

Figure 1: Structures of polygodial (**1**), isopoligodial (**2**), drimenol (**3**) and isodrimenol (**4**) obtained by catalytic hydrogenolysis of isodrimendiol (**5**) prepared by reduction of **2** with sodium borohydride.

Compounds **1**-**4** were evaluated for their antifungal activity against a panel of human pathogenic fungi by the microbroth dilution assay recommended by CLSI (Clinical and Laboratory Standards Institute).[3]

Results showed that **1** was active against 6/9 tested fungi with MICs between 3.9 to 62.5 µg/mL, meanwhile **2** was active against 4/9 tested fungi (MICs 62.5 µg/mL), indicating that the aldehyde function in C-9β makes compound **1** more active and with broader spectrum of action than **2**.

Otherwise, compound **3** was active against 4/9 tested fungi with MICs between 62.5 to 125 µg/mL, while **4** was active against 6/9 tested fungi with MICs between 31.2 to 250 µg/mL, indicating that the alcohol function in C-9α makes compound **4** more active and with broader spectrum of action than **3**.

These results confirm the importance of C9 absolute configuration for the antifungal activity of sesquiterpene dialdehydes and monoalcohols.

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Acknoledgments: CONICET, ANPCyT, UNR.

Keywords: Sesquiterpenes, antifungal, semisynthesis.

Influence of chitosan molecular weight on its antifungal activity

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INTRODUCTION. Vulvovaginal candidosis (VVC) is a common clinical manifestation of *Candida* infections and is the second most frequent vaginal infection, after vaginal bacteriosis. *Candida albicans* is the common species isolated from VVC and recurrent cases, followed by *C. glabrata*, *C. tropicalis* and *C. krusei*.
VVC is usually treated very effectively with azoles. In contrast, patients with recurrent *Candida* spp infection are difficult to manage. Most gynecologists feel that the control of VVC/RVVC will require both systemic and local therapeutic approaches also involving new antifungal drugs and strategies.

Natural products have been the major source of drugs for centuries and continue to have a central role in the discovery and development of new pharmaceuticals. Chitosan, a natural polysaccharide resulting from alkaline deacetylation of chitin obtained from crustaceans, insects and fungi, has been widely used as pharmaceutical excipient. Being a non-toxic and muco/bioadhesive polymer, chitosan is an excellent carrier for mucosal drug delivery system, improving the adhesion between drug formulation and the mucosal tissue. Chitosan hydrogels and microcapsules are two available systems with possible application on mucocutaneous infections. The polymer also showed to be effective against a wide range of microorganisms. Its antimicrobial effect appears to be dependent on its molecular weight and degree of deacetylation, since its mechanism of action seems to be related with the protonated amino groups. We have previously reported the anti-*Candida* activity of a medium molecular weight chitosan [1]. Being itself an antifungal compound, chitosan may represent an interesting product to be used on vaginal delivery systems for the treatment of vulvovaginal candidosis.

In this study we present the antifungal activity of two hydrogels containing chitosans with different molecular weight (low and high molecular weight).

STUDY DESIGN. Eleven clinical and type strains of *Candida* were studied: 6 *C. albicans* (1 ATCC strain, 5 clinical strains); 3 *C. glabrata* clinical strains; 2 *C. tropicalis* clinical strains. A high molecular weight chitosan (HMW 310000-350000 Da), 76% deacetylation degree (SIGMA, Portugal) and a low molecular weight chitosan (LMW 50000-190000 Da), 92% deacetylation degree (SIGMA, Portugal) were used. 4% (m/m) high molecular weight chitosan hydrogel (HCH) and a 5% (m/m) low molecular weight chitosan hydrogel (LCH) were prepared dissolving the chitosan on 2% acetic acid solution (Aldrich). The anti-*Candida* activity of chitosan hydrogels (CH) was studied according to CLSI reference M27-A3 protocol. Minimal inhibitory concentrations (MIC) were determined after 48 hours of incubation at 37ºC. Yeast growth was visually compared for each concentration with the control sample. Additional controls were performed with RPMI medium containing acetic acid 2%. All determinations were performed in duplicate.

RESULTS. HCH and LCH were active against all *Candida* spp tested, *C. albicans* and *C. glabrata* being the less sensitive strains. MIC for HCH against *C. glabrata* was 6.0-7.5 mg/mL and for LCH was 7.5 mg/mL. Similar difference was achieved for *C. albicans* (HCH 2.5-5.0 mg/mL and LCH 5.0 mg/mL). For *C. tropicalis* MIC was the same for both CH (1.25 mg/mL). The effect was not influenced by acetic acid as all tested strains and respective controls exhibited similar growth in its presence or absence.

CONCLUSIONS. Chitosan molecular weight has some influence on its activity against *Candida* spp. In some extent, the higher deacetylation of the LMW chitosan may also had some contribution to the observed slight difference of these two hydrogels activity. However, being more difficult to manipulate due to its higher viscosity, HMW chitosan appear to have low or no advantage to be used as hydrogel for vulvovaginal application. LMW chitosan, on the other hand, presents similar antifungal activity and meets technological advantages that make of it a more interesting product for drug delivery systems regarding vaginal application on candidosis.

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Inhibitory activity of polyhydroxyflavanones against methicillin-resistant *Staphylococcus aureus* (MRSA)

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is reputedly known as one of the most problematic clinically relevant pathogens at present and ranks as one of the most difficult bacteria to treat in patients and to eradicate in a hospital environment. This is due to its genetic plasticity and rapid evolution of drug-resistance and virulence. Studies have shown that plant flavanone has good antibacterial properties against many clinically important bacteria including MRSA.

Previously, naringenin-4'-methyl ether, was isolated from a local medicinal plant. However, it only displayed weak inhibitory activity against a panel of multidrug-resistant MRSA isolates with minimum inhibitory concentration (MIC) value of more than 500 µg/ml. In this study, four structurally similar derivatives were synthesized and evaluated using MIC value determination assay to further investigate the structure-activity-relationship (SAR) of this flavanone compound.

Multi-step chemical reactions (methylation, acethylation, oxidation and aldol condensation) were used to synthesized the flavonoids derivatives and characterized spectroscopically by using infra red (IR), Nuclear Magnetic Resonance (Proton and Carbon) and Mass Spectrometry. Upon completion, all of the compounds were subjected to the MIC assay.

Compound 4',5,7-trihydroxy-3'-prenylflavanone was found to be the most potent with the lowest MIC value of 31.3 µg/ml and followed by both 4',5,7-trihydroxyflavanone (naringenin) and 4'-ethoxy-5,7-dihydroxyflavanone with MIC value of 125 µg/ml. Eriodictyol or 3',4',5,7-tetrahydroxyflavanone however exhibited similar weak inhibitory activity as naringenin-4'-methyl ether with MIC value of more than 500 µg/ml.

The structure-activity-relationship (SAR) study suggested that inhibitory activity was governed to a greater extent by the presence phenolic groups and certain degree of lipophilicity is required for the activity of the flavanones. In addition, the modification of the functional groups on position C-3' and C-4' of the chemical structure seems to play an important role in affecting the inhibitory ability of each compounds.

Nevertheless, ensuing toxicology assessments are needed prior its development for potential therapeutic applications. In conclusion, synthetically-produced bioactive derivatives based on naturally-occurring compounds in plant may play an important role in the quest for new and effective anti-MRSA agents.

Keywords methicillin-resistant Staphylococcus aureus (MRSA); synthetic compounds; polyhydroxyflavanones; MIC assay

Introduction of sub/supercritical fluid extraction as a new sample-preparation procedure for isolation and identification of a pharmaceutical from biological fluids: Application to disposition kinetics

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Since its commercial development in the early 1990s, supercritical fluid extraction (SFE) has attracted considerable attention as a sample-preparation procedure. However, other different sample preparation procedures, including precipitation, liquid- and/or solid-phase extraction, still remain in popular use in biological fluids. In this investigation, SFE was introduced to isolate and identify orbifloxacin (OBFX), a fluoroquinolone antimicrobial, from plasma and milk. Four parameters, including the temperature and the pressure of supercritical fluid, modifier ratios and dynamic extraction time, were evaluated and optimized to obtain the best yield of the analyte from the biological fluids. Determinations of the OBFX in the extracts were carried out using high-performance liquid chromatography (HPLC-FLD). The optimum conditions of the extraction process that yielded the maximum extraction efficiencies (recovery %) of the analyte were 150°C vs. 60°C, 250 kg/cm^2, 30% vs 35% methanol, and 40 min vs. 20 min, for plasma and milk, respectively. The linearity (r^2) of the calibration curves based on five concentrations measured in 3-fold, as well as the instrument limits of detection and quantitation (LOD/LOQ) were evaluated. Good linearity (at least $r^2 \geq 0.999$) of the calibration curves was obtained over the range from 0.2 to 0.01 µg mL^{-1}. The method gave quite good extraction efficiency (recovery rate: 74.2–127.73%) and precision (RSDs: 1.64–20%). The instrumental LOD and LOQ values were 0.004 µg mL^{-1} vs. 0.01 µg mL^{-1}, or 0.006 µg mL^{-1} vs. 0.02 µg mL^{-1}, for plasma and milk, respectively. The method was successfully applied to estimate the pharmacokinetic variables in lactating does (n=7) following intravenous (IV) and intramuscular (IM) administration of OBFX at a dose rate of 2.5 mg kg^{-1} bwt. To the best of our knowledge, this is the first time SFE has been applied to isolate an antimicrobial agent from biological fluids. This method is promising for clinical applications and for pharmacokinetic studies of various pharmaceuticals in biological fluids.

Keywords: Biological fluids; Plasma; Milk; Small ruminants; Supercritical fluid extraction; Fluoroquinolones

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Isopropanol and Potassium Acetate Greatly Enhance the Antimicrobial Activity of Glutaraldehyde

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Current high level disinfectants as used for the disinfection of heat-sensitive, semi-critical devices such as endoscopes, have various deficiencies of antimicrobial activity and patient safety. Alkaline glutaraldehyde has a label claim of 45.0 min at 25ºC determined by limited kill of mycobacteria. By concensus, it is often used for 20.0 min at 20ºC, or even for 10.0 min. In 2008, the National Health and Sanitary Surveillance Agency (ANVISA) of Brazil reported more than 2000 cases of serious wound infections due presumably to glutaraldehyde-resistant *Mycobacterium massiliense*. *M. chelonae* var. *abscessus* has also been identified as glutaraldehyde-resistant. The kill of spore-forming *Bacillus subtilis* is also very slow within the brief exposure time used for disinfection of endoscopes, and possibly the survival of such bacteria in the channels of endoscopes could be one cause of biofilms. It is very difficult to disinfect an endoscope after a biofilm has formed.

Ortho-phthalaldehyde (OPA) does not kill *B. subtilis* in any practical exposure time, again raising the possibility that surviving cells of *B. subtilis* could form a biofilm. The kill of mycobacteria by OPA within 5.0 or 12.0 min might be limited to cells of *M. terrae* in suspension, and much slower when mycobacteria are dried onto a surface. OPA is relatively insoluble, and thus difficult to rinse from equipment, and the labels of OPA carry a warning against use with bladder cancer patients, where repeated and frequent exposures to a residue of OPA on cystoscopes have led to cases of anaphylactic shock.

Peracid formulations of hydrogen peroxide and peracetic acid are fast and broad-spectrum high level disinfectants. However the mode of action is for the peracetic acid to break down to form hydroxyl ions, and thus the use and re-use life of peracids is relatively brief and thus comparatively expensive.

We tested many combinations of glutaraldehyde, alcohols and salts, and discovered that very low concentrations of isopropanol in the range of 10% to 20% w/w killed mycobacteria in suspension and as dried onto inert surfaces within 10.0 min at 20ºC. Potassium acetate salts also enhanced the mycobactericidal activity of glutaraldehyde, as well as the sporicidal activity, especially in combination with isopropanol. These chemicals are very soluble in water, and thus can be rinsed easily from surfaces. The chemistry is stable and can be used and re-used for at least 14 days depending on the volume of devices to be disinfected. This new FDA-cleared-to-market high level disinfectant is called Aldahol High Level Disinfectant. Comparative antimicrobial data will be shown of glutaraldehyde, OPA, and Aldahol High Level Disinfectant. Aldahol killed 8 $\log_{10}$ of *M. massiliense* within 5.0 min at 25ºC. #

Large-scale purification of nigrin b, a ribosome-inactivating protein from *Sambucus nigra* L.

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Ribosome-inactivating proteins (RIPs) are polynucleotide N-glycosidases with antifungal and antiviral activities. They are composed by either one (type 1 RIPs) or two (type 2 RIPs) polypeptide chains. Nigrin b, a non-toxic type 2 RIP present in the bark of elderberry (*Sambucus nigra* L.), is of great interest for pharmacological applications in the construction of immunotoxins and conjugates for experimental therapy. The aim of the present work was to find the best source of protein, and the development of a new, much more efficient procedure for preparing the protein in order to test its potential antiviral and antifungal activities, and assessment of its biochemical and biological characterization (protein synthesis inhibition in cell-free systems, rRNA N-glycosidase activity, cytotoxicity and sugar-binding ability). The highest amount of nigrin b was found in the secondary bark of spring and summer plants. Nigrin b displayed a low and temperature-sensitive affinity for a polysaccharide matrix derived from Sepharose 6B by treatment with acid, which has been used to date for the preparation of nigrin b. The core of the present improved procedure involved ion-exchange chromatography instead of affinity chromatography. The protein isolated in this way presented not only maintained full anti-ribosomal activity but also display a powerful antiviral action on Tobacco mosaic virus (TMV). Such action was dependent on pH and showed a maximum at pH 4. Furthermore, it has been shown that nigrin b is resistant to pepsin (pH 1.0) degradation at least for 60 min at 37^0C.

Keywords Ribosome-inactivating protein; nigrin b; ricin; *Sambucus nigra*; antiviral protein; antifungal protein

Macromolecule-peptide fusions as tools for crystallization of glycopeptide antibiotics.

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The growing problem of bacterial antibiotic resistance increases the demand for conformational studies of antibiotics binding their targets. Glycopeptide antibiotics bind a d-Alanine d-Alanine peptide that is part of the peptidoglycan mesh in Gram-positive bacteria, thereby inhibiting cell wall biosynthesis. However, crystallographic studies examining the details of this interaction have been limited due either to difficulties in the crystallization of antibiotic-ligand complexes or obtaining phases to solve the structure. We have constructed protein fusions to a bacterial cell wall mimetic peptide for use as tools in glycopeptide antibiotic crystallographic studies. Such tools should facilitate crystallization, via the surfaces of the protein partners, and provide initial phases, via molecular replacement, to eventually solve the structure. We present a high-resolution crystal structure of an MBP-peptide fusion construct bound to ristocetin, a glycopeptide antibiotic. Here we show using surface plasmon resonance that our fusion constructs bind glycopeptide antibiotics with appropriate affinities. Our results demonstrate the utility of macromolecule-peptide fusions as tools to assist the conformational studies of antibiotics binding their targets. The information provided by these studies will contribute to the design of new drugs to overcome bacterial resistance.

Keywords glycopeptide; ristocetin; crystallography; structure

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Nalidixic acid-eluting polypropylene functionalized at the surface with stimuli responsive polymers

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Morbidity and mortality associated to device-related infections have become a relevant source of sanitary and economic worries. The surfaces of medical devices are not exempt of being colonized by microorganisms, resulting in infection focuses that are hardly accessible to antimicrobial drugs. The aim of this work was to functionalize the surface of polypropylene (PP), which is a component of a wide range of medical devices (e.g., catheters, hernia meshes, sutures), with a hydrogel layer able to interact with an antimicrobial agent and to sustain its delivery without detriment of the hemocompatibility of PP.

PP films were grafted with N-isopropylacrylamide (NIPAAm) and N-(3-aminopropyl) methacrylamide hydrochloride (APMA) applying a pre-irradiation method, which consisted in i) exposition of PP to ^{60}Co γ-source (Gamma beam 651 PT, MDS Nordion USA) in the presence of air, at room temperature. Dose rate of 10 kGy/h and pre-irradiation dose of 50 or 80 kGy were applied; followed by ii) immersion in glass ampoules which contained aqueous solutions of NIPAAm/APMA 1/0.5 or 1/1 M for obtaining PP-g-(NIPAAm-r-APMA), or 1 M NIPAAm solution in order to prepare PP-g-NIPAAm. The extent of grafting was modulated by the time of immersion in the monomers solution.

The grafting composition was analyzed recording FTIR-ATR spectra. PP-g-(1NIPAAm-r-0.5APMA) exhibited the temperature-responsiveness of PNIPAAm, while the grafting with a greater content in APMA led to that PP-g-(1NIPAAm-r-1APMA) remained highly swollen at 37°C. Pristine PP films did not adsorb nalidixic acid once immersed in the drug solution. The capability of PP-g-NIPAAm to load the antimicrobial agent was also minor, just 0.4 $\mu g \cdot cm^{-2}$. By contrast, the copolymerization of NIPAAm with APMA increased two orders of magnitude the ability of the grafted PP to take nalidixic acid up (up to 36 $\mu g \cdot cm^{-2}$). These findings suggest that the drug does not interact with the NIPAAm moieties but electrostatically with the quaternized amine groups of the APMA. In fact, a linear correlation was observed between the amount of nalidixic acid loaded and the amount of APMA in the grafted copolymer. The greater the content in APMA on the PP surface, the slower the release rate in phosphate buffer pH 7.4. Coating with carboxymethyl-dextran of some drug-loaded films led to minor drug unloading while remarkably high amounts of dextran were deposited (up to 500 $\mu g \cdot cm^{-2}$). This coating did not significantly modify the drug release rate neither the hemocompatibility of the PP-g-(NIPAAm-r-APMA) films, which was per se very good. Drug-loaded films remarkably inhibited the growth of *Escherichia coli* in in vitro microbiological tests.

Keywords: polypropylene; nalidixic acid; N-isopropylacrylamide; N-(3-aminopropyl) methacrylamide; controlled release; γ-ray irradiation; antimicrobial surface; medicated medical device.

Natural Product-Derived Antifungals: From (-)Incrustoporin to 5-Alkylidene-3-(haloaryl)-2,5-dihydrofuran-2-ones

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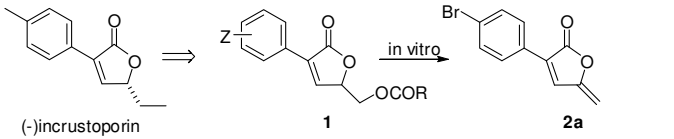
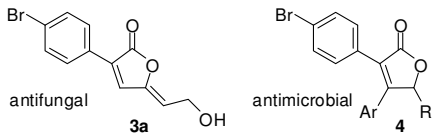
Antifungally highly active 5-acyloxymethyl-3-(haloaryl)-2,5-dihydrofuran-2-ones[1] (**1**) derived from (-)incrustoporin have been found to produce the corresponding 5-methylene derivatives such as **2a** under in vitro assay conditions[2]. As the latter compounds possessed nearly the same activities as their precursors **1** (eg. **2a**: IC_{80} 0.49 to 15.62 μmol/L against *Candida spp.*, 1.95 to 7.81 μmol/L against *A. fumigatus*), their formation was the reason for the observed antifungal activity of the former.



Following the structure of **2a**, we have prepared a series of novel 5-alkylidene furanones via Pd-catalyzed cyclisation of enynoic acids. Notably, their antifungal activities decreased in comparison to **2a**, with the exception of furanone **3a** bearing an allylic hydroxyl on the exocyclic double bond. Attachment of a second aryl moiety to C4 led to antifungally inactive compounds **4**. These C4-arylated derivatives, however, possessed interesting antimicrobial activities (in the order of units of μmol/L) against *S. aureus*.



Apart from an overview of the structure activity-relationships, the development of the lactone-based antifungals will be discussed with emphasis on the application of Pd-mediated reactions to the syntheses of the target butenolides.

[1] Pour, M.; Špulák, M.; Buchta, V.; Kubanová, P.; Buchta, V.; Vopršalová, M. et al J. Med. Chem. 2001, 44, 2701.
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Acknowledgement. Generous support from Charles University (project No. SVV/2010/261/001) and the Ministry of Education oft he Czech Republic (projects Nos. 1M0508 and MSM 0021620822) is gratefully acknowledged.

Keywords antifungals;antimicrobials;butenolides;furanones;Pd-catalysis

Novel Heterocyclic Building Blocks for Bioactive Compounds: Direct Activation of C-H Bond in Tetrazoles

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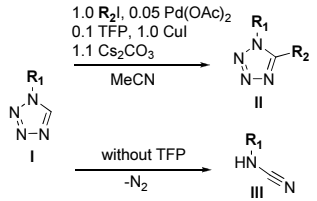
New heteroaryl compounds continuously emerge as lead structures for novel pharmaceuticals. Among them, 1,5-disubstituted tetrazoles occupy an important place because of being able to act as NAD(P)H oxidase inhibitors, glucokinase activators, hepatitis C virus (HCV) serine protease S3 inhibitors, calcitonine gene-related peptide receptor antagonists and antimigraine agents.[1] Their preparation from 5-substituted tetrazoles is accompanied by the formation of regioisomers which are often difficult to separate, while syntheses starting with acyclic precursors and using classical reagents suffer from long reaction times, high temperatures, low yields, difficult work-up and use of toxic reagents.[2,3]

Thus, we became interested in exploring a more attractive possibility of a direct activation of the C5-H bond, since these reactions constitute an alternative to traditional cross-couplings. Since 1-substituted tetrazoles (**I**) can be prepared in a one-pot process from primary amines, orthocarboxylic acid ester and sodium azide[4], subsequent C-H bond activation would enable a rapid entry into a range of 1,5-disubstituted tetrazoles from primary amines. Similar to C2 in imidazoles and C8 in purines, tetrazole C5 is surrounded by two nitrogens. Our initial attempts were therefore based on an attractive phosphine free protocol developed by Hocek et al[5] for the intermolecular arylation of C8 in purines, however, just a trace amount of the desired tetrazole (**II**) were obtained, while subst. cyanamide (**III**) arising from the fragmentation of the tetrazole ring was the only isolated product. Interestingly, the addition of a 10 % molar amount of tris(2-furyl)phosphine (TFP) raised the yield of 1,5-disubstituted tetrazoles (**II**) to excellent.



In conclusion, we have explored the conditions for the direct C-H arylation/alkenylation of 1-substituted tetrazoles, and developed a high-yielding protocol to achieve this transformation. The reaction is widely applicable and opens up a short and reliable route towards a range of 1,5-disubstituted tetrazoles.

Acknowledgement. This work was supported by the Czech Science Foundation (project No. 203/07/1302) and the Ministry of Education, Youth and Sports of the Czech Republic (projects Nos. 1M0508 and MSM0021620822).

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Keywords tetrazoles; Pd-catalyzed cross-coupling reaction

Overcoming Nature's Potential to Develop Novel Tetracyclines

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Tetracyclines (TCs) are a large group of medically-important antibiotics with a common basic structure of four linearly fused six-membered rings. They are produced by several genera from the order Actinobacteria; some examples are tetracycline, chlortetracycline, oxytetracycline, and demethylchlortetracycline, synthesized by the type II polyketide synthase (PKS) multi-enzyme complexes. A number of potent antibacterial TCs have been generated using a semi-synthetic approach such as minocycline, doxycycline and the novel tigecyclin, which has recently proved to be a very efficient anti-infective. Currently used TCs act at the ribosome and interfere with bacterial protein synthesis. These anti-infective compounds were amongst the first broad-spectrum antibiotics and their intensive use unfortunately led to widespread microbial resistance. Interestingly, a small group of tetracycline analogs has recently been identified that do not target bacterial ribosome. Instead, they have bactericidal rather than bacteriostatic activity, and are active even against the tetracycline-resistant strains, thus representing an interesting new group of TC derivatives with unexplored potential. We have cloned, sequenced and characterized a novel gene cluster encoding the unusual TC antibiotic chelocardine. The unusual structure of this tetracycline analog provides an opportunity for the development of new tetracycline molecules.

Keywords: tetracyclines, type II polyketide, antibacterial activity

Overexpression of the putative allylmalonyl-CoA extender unit enhances the FK506 productivity

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FK506 (tacrolimus) is a polyketide compound with immunosuppressive and neurotrophic activities. Since the discovery of FK506 from the culture broth of soil bacteria *Streptomyces tsukubaensis* in 1987, it has become a clinically important drug and currently registered for use as immunosuppressant after organ transplantation. FK506 and FK520 are biosynthetically related polyketides. From a biosynthetic viewpoint, FK506 has a structurally unique feature compared with FK520 and FK506 is the only polyketide which carries an allyl side chain on carbon 21. *Streptomyces* sp. MJM7001, FK506 high-producer, was produced from *S. tsukubaensis* No.9993 by UV-mutagenesis. The FK506 gene cluster from *Streptomyces* sp. MJM7001 has been sequenced and we identified additional 9 genes, which are not existed in the FK520 gene cluster. Among these 9 genes, only 4 genes (*allA, allB, allC, allD*) were found in other FK506 gene clusters. The gene expression level of *allA, allB, allC, allD* from *S. tsukubaensis* No.9993 and MJM7001 was checked by RT-PCR and it was higher in *Streptomyces* sp. MJM7001. So, we predicted that these 4 genes are related to an allyl side chain forming in FK506 biosynthesis. The 4 genes, *allA, allB, allC, allD*, were cloned into the high copy number vector (pWHM3) and overexpressed in *S. tsukubaensis* No.9993. The FK506 production of overexpression mutant was enhanced up to 40%.

Keywords : FK506, *S. tsukubaensis*, FK506 gene cluster

Poly(hexamethylene biguanide)-functionalized paramagnetic nanoparticles as biocidal agents against Gram-positive bacteria

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Magnetic nanoparticles functionalized with antimicrobial compounds may offer a green and cost-effective approach to the eradication of pathogenic microorganisms from contaminated water sources, aqueous media or soil. Compared to the indiscriminate addition of antimicrobial chemotherapeuthic agents that disperse in the environment and remain in it until degradation, magnetic particles bearing bactericidal substances may combine an efficient capture of a germ by the particles, followed by an efficient removal of the particles (with attached dead cells) from the medium by simple activation of a magnetic field. We have previously observed that magnetite nanoparticles functionalized with poly(hexamethylene biguanide) (PHMBG) can be captured, together with germs bound to the particle surface, by a high gradient magnetic separation (HGMS) process, and that the microorganism DNA could be extracted and analyzed by real-time polymerase chain reaction. Importantly, the PHMBG-functionalized particles were able to act as lipopolysaccharide (LPS)-sequestering agents from Gram- bacteria and to kill *Escherichia coli* at concentrations significantly lower than those toxic for mammalian cells. Therefore, these particles may enable not only in-situ biodefense, but also a method of monitoring the presence of dangerous germs in aqueous habitats.

In the context of developing broad-range biocidal magnetic nanoparticles, the aim of the present study was to evaluate if PHMBG-functionalized particles, prepared using two different synthetic routes, were also useful against Gram+ microorganism. Poly(hexamethylene biguanide) and polyethyleneimine-modified magnetite (PHMBG-PEI-M) particles, and magnetite nanoparticles encapsulated with silica and modified by pol(hexamethylene biguanide) (PHMBG-M/SiO_2) were prepared. The minimum inhibitory concentrations (MIC) of common human Gram+ pathogens (*Staphylococcus aureus* and *Staphylococcus epidermidis*), but also of cultured fishes (*Streptococcus phocae* and *Lactococcus garviae*), were determined and compared to the MICs of Gram- representatives *Escherichia coli* and *Pseudomonas aeruginosa*. Then, the binding of the PHMBG-functionalized particles to the D-Ala-D-Ala intermediates in the peptidoglycan of the Gram+ bacteria cell wall was analyzed to gain an insight into the mechanism involved in the inhibition of the bacterial growth. The peptide D-Ala-D-Ala-D-Ala labeled with 5-carboxyfluorescein (5-FAM$(DA)_3$) was used as a model peptide. Fluorimetric assays were carried out to investigate the binding of the peptide and competitive displacement by *Staphylococcus aureus*. From the equilibrium binding data, it was shown that many millions of peptide molecules can be adsorbed by one gram of particles, confirming that both PHMBG-PEI-M and PHMBG-M/SiO_2 possess a high affinity for peptidoglycans.

Keywords: magnetic particles; bactericidal effect; peptidoglycan; poly(hexamethylene biguanide); bioremediation.

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Pyridine derivatives active against multi-drug resistant *M. tuberculosis* strains

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Tuberculosis (TB) still remains a major public health problem. More than 2 billion people are infected with *Mycobacterium tuberculosis*, the microbes that cause TB. 1 in 10 people infected with TB bacilli will become sick with active TB in his or her lifetime. Very high risk is among HIV positive people. TB is a disease of poverty affecting mostly young adults in their most productive years. In 2008 there were 9.4 million new TB cases and 1.8 million people died. Multidrug-resistant TB (MDR-TB) is a form of TB that does not respond to the standard treatments using first-line drugs. The proliferation of multidrug-resistant (MDR) strains and the high susceptibility of HIV-infected persons have created much scientific effort in developing new antimycobacterial agents to both treat *Mycobacterium tuberculosis* strains resistant to existing drugs, and shorten the duration of short-course treatment to improve patient compliance.

The research in our laboratories has been directed towards the search for new compounds with antimycobacterial activity for several years. As a part of this programme, we synthesized pyridine derivatives. The screening of new prepared compounds revealed the significant activity against *Mycobacterium tuberculosis*. The rising emergence of multi-drug-resistant strains of *M. tuberculosis* prompted us to investigate the activity of these compounds also against MDR strains.

The compounds were evaluated against *M. tuberculosis* CNCTC My 331/88 and several strains of MDR *M. tuberculosis* using the micromethod for the determination of the minimum inhibitory concentration. Antituberculotic activities were determined in Šula´s semisynthetic medium (SEVAC, Prague). The compounds were added to the medium in dimethyl sulfoxide solutions. The following concentrations were used: 500, 250, 125, 62, 32, 16, 8, 4, 2 and 1 μmol/L. The MICs was determined after incubation at 37 °C for 14 and 21 days.

The MIC values are varying within the range of 1-62 μmol/L against all tested strains. There are no significant differences in susceptibility of sensitive and multidrug-resistant strains of *M. tuberculosis*. Comparable susceptibility of MDR and sensitive strains of *M. tuberculosis* indicate that there is no cross resistance with current antituberculosis drugs. Structure-activity relationship within the prepared compounds will be discussed.

Keywords *Mycobacterium tuberculosis,* multidrug-resistant strains, pyridine derivatives

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Rational *De novo* Design of α-Helical Antimicrobial Peptides with Exceptional Therapeutic Indices Against Clinical Isolates of Gram-negative Pathogens, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: Optimizing charge, "specificity determinants," hydrophobicity, type and location of hydrophobes

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Acinetobacter baumannii and *Pseudomonas aeruginosa* are two increasingly important hospital-associated gram-negative pathogens and are extremely difficult to treat because of the ever increasing resistance to classical antibiotics. Multi-drug resistant strains of *Acinetobacter baumannii* have rapidly emerged in all parts of the world. Thus, there is an urgent need for new classes of compounds with different modes of action compared to classical antibiotics. Antimicrobial peptides (AMPs) may represent such a new class and have become important candidates as potential therapeutic agents.

In previous studies we introduced the concept of a "specificity determinant" where substitution of a lysine residue in the center of non-polar face of 26-residue amphipathic α-helical AMPs dramatically reduced toxicity and increased the therapeutic index [1-4]. We also showed that our L- and D- enantiomeric AMPs had equivalent biological and biophysical properties and their sole target was the bacterial membrane [2]. The excellent stability of the D- enantiomers to proteolysis highlights their potential as clinical therapeutics.

In this study, we introduced a second lysine specificity determinant in the center of the non-polar face to further reduce toxicity and at the same time allow for increased hydrophobicity. We then investigated the role of systematic increases in hydrophobicity, varying the type of hydrophobic side-chain (Leu, Ile, Phe and Trp) and the location of the hydrophobes within the non-polar face as well as optimizing the location and number of positively charged residues on the polar face of these molecules. We also investigated the effect these systematic changes on the biological and biophysical properties of these AMPs. We evaluated these peptide analogs for their antimicrobial activity against eleven clinical isolates of *Acinetobacter baumannii* and six clinical isolates of *Pseudomonas aeruginosa*, hemolytic activity to human red blood cells, structure in aqueous and hydrophobic media, overall hydrophobicity and self-association ability. The rational design approach used in this study allowed us to optimize the location and net positive charge on the polar face and the location and type of hydrophobe on the non-polar face in conjunction with our two specificity determinants (K13 and K16) on the non-polar face to achieve unprecedented biological properties for these *de novo* designed AMPs.

Our previous lead compound known as V13K [1,2] had an antimicrobial activity (geometric mean of MICs) of 1.1 μM, hemolytic activity (HC_{50}) of 140.9 μM and a therapeutic index of 128.1 against *Acinetobacter baumannii*. To our surprise, we obtained a new antimicrobial with outstanding properties, an antimicrobial activity (geometric mean of MICs) of 0.4 μM, hemolytic activity (HC_{50}) of 1,342 μM and a therapeutic index of 3,355, 26-fold better than our previous lead peptide. We will discuss the rational design approach used to develop this new exceptional peptide.

Keywords: antimicrobial peptides, rational design, specificity determinants, antimicrobial activity, hemolytic activity, therapeutic index

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Screening a novel compound library for inhibitors of *Mycobacterium marinum* and further investigation of unexpected phenomena

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Mycobacterium marinum (*M.marinum*) an ectotherm pathogen is the closest genetic relative of *Mycobacterium tuberculosis* and is therefore a useful category II indicator species for possible activity against TB. A novel synthetic compound library of 720 compounds was screened for activity against *M.marinum* using a high throughput microplate alamar blue assay. Minimum inhibitory concentration (MIC) values were determined for the active compounds. Upon analysis of the MIC data it was suspected that some of the compounds were causing interference to neighbouring wells in the microplate. Further experiments were carried out to determine if the compounds were interfering with the alamar blue REDOX indicator or if the compounds were active against *M.marinum* as volatiles in the gaseous phase.

Methodology and experimental data will be presented and discussed.

Keywords *Mycobacterium marinum*; *Mycobacterium tuberculosis*; active compound; antibacterial; volatile.

Stereochemical involvement of glycans in microbes; A theoretical approach

MUHAMMAD RAMZAN MANWAR; NASIR-UD-DIN,ABDUL RAZAQ AND LUBNA CHUDHARY

HAJI NAZIR KARYANA STORE AHMED STREET NO. 10 NEW SHAH KAMAL COLONY ICHHRA LAHORE, PAKISTAN.

Complex carbohydrate structures are the most common receptor for microbes adhesions and infections for mammalian cells. The glycan-protein interaction for the recognition of sugars sequences within glycan chain is one of characteristic property of microbes. A microbial ligand may show different binding abilities for the isoreceptors depending upon steric hindrance and stereochemical conformations of terminal and vicinal residues upon contact to the binding epitope. The current study relates the pathological involvement of sugars like Gal (Galactose) and GalNAc (*N*-acetylgalactosamine) with their structural chemistry in glycan chains. Glycans having Gal/ GalNAc at the non-reducing and reducing end have been documented to play vital role in microbial recognition and their cellular adhesion. The stereochemistry of the sugar residues with their particular features like sequence and anomeric linkages is involved in various pathological events. This type of structure function relationship is not documented in many current databases. This study approach is also helpful for the anti microbial drug modeling.

Strong *In Vitro* Activity of Two New Rifabutin Analogs against Multidrug-resistant *Mycobacterium tuberculosis*

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The World Health Organization has documented an increasing problem of multidrug-resistant tuberculosis (MDR-TB).[1] Two new rifabutin analogs,[2] called rifastur 1 (RFA-1) and rifastur 2 (RFA-2) showed high *in vitro* activity against susceptible and resistant strains of *M. tuberculosis*, including MDR-TB. Seventy-nine *M. tuberculosis* strains (63 clinical isolates and 16 reference strains) were analyzed and were found to be highly susceptible to the rifastures (MIC's ≤0.02 µg/mL). All of the 23 MDR-TB or rifampin-resistant strains tested showed strong in vitro susceptibility to the rifastures, with MICs of 0.5 µg/mL compared to 50 µg/mL for rifampin and 10 µg/mL for rifabutin. Molecular dynamic studies suggest that the elevated bioactivity of RFA-1 and RFA-2 against the rifamycin-resistant TB is due to tighter binding to RNA polymerase resulting in the formation of a more stable protein-inhibitor complex.[3]

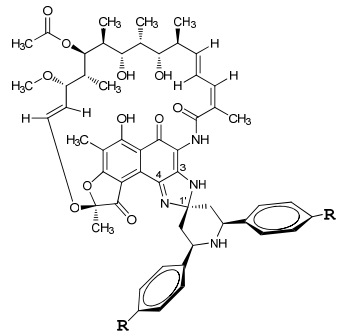

Figure 1. Chemical structure of RFA-**1** (R = H) and RFA- **2** (R = F).

Keywords Rifamycins,Rifampin, Rifabutin, Rifastures, *M. tuberculosis, MDR-TB, RNA polymerase.*

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Structure-based drug design targeting the essential response regulator WalR of *Staphylococcus aureus*.

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A two-component system comprised of an HK (histidine kinase) designated WalK (YycG) and its cognate RR (response regulator) designated WalR (YycF) is specifically conserved in Gram-positive bacteria including

B. subtilis and *S. aureus*, and it is essential for their growth. The essential nature of this signaling system makes it an attractive target for developing novel antibiotics (1).

To gain insight into the structure-based function of WalR, we have determined the X-ray crystal structures of both the N-terminal dimerization domains (Fig. 1) and C-terminal DNA binding (Fig. 2). The results suggest that WalR is a typical response regulator of the OmpR/PhoB subfamily with a characteristic winged helix-turn-helix DNA-binding domain. WalR controls the expression of WalR regulon genes by forming a head-to-head dimer of the receiver domains, using the conserved α4-β5-α5 face, paired with a head-to-tail dimmer of the winged helix-turn-helix motifs that bind to the tandem DNA repeats (WalR box) (2). Based on this structure, we have developed a screening method to isolate the inhibitor (named walrycin B) targeting WalR (3). Walrycin B regulated simultaneously the expression of *walR* regulon genes, leading to long aseptate filaments of *B. subtilis* and large aggregates of *S. aureus* while also causing a bactericidal effect. Furthermore, a docking model between WalR and walrycin B was proposed. The results suggest that walrycin B is the first antibacterial agent targeting WalR in *B. subtils* and *S. aureus*.

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Keywords Two-Component System; WalR; Response Regulator; X-ray Crystal Structure; *Staphylococcus aureus*; walrycin;

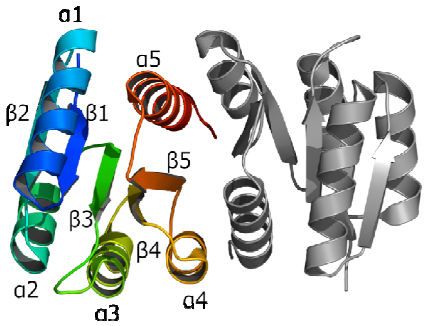

Fig. 1. N-terminal dimerization domain of WalR (2ZWM).

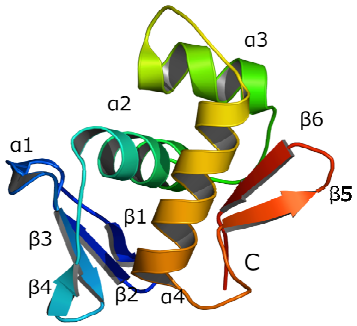

Fig. 2. C-terminal domain of WalR (2ZXJ).

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Study on Antimicrobial Activity of Synthetic Thioflavones

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In this study, the antimicrobial activities of 8 synthetic thioflavones (thioflavone, *O*-methyl-thioflavone, *P*-methyl-thioflavone, *O*-chloro-thioflavone, *m*-chloro-thioflavone, *p*-chloro-thioflavone, *O*-methoxy-thioflavone, *p*-methoxy-thioflavone, 3,4-dimethoxy-thioflavone and 3,4,5-trimethoxy-thioflavone) against 3 Gram (+) bacteria (*Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus*), 7 Gram (-) bacteria (*Aeromonas hydrophila*, *Salmonella choleaesuis*, *Salmonella enterica*, *Serratia marcescens* and *Vibrio parahaemolyticus*) and Fungi, *Rizopus* sp. were investigated.

The antimicrobial activities of synthetic thioflavones were evaluated using broth microdilution assay and agar diffusion assay and in this way determined MIC (minimum inhibitory concentration) and MBC (mibactericidal concentration). MIC values for tested bacteria which were sensitive to the all used synthetic thioflavone in the range of 31.25-500 μg/mL. All synthetic thioflavones were determined MBC values to be 250-500 μg/mL against *Vibrio parahaemolyticus*, and MBC of Thioflavone and *O*-methyl-thioflavone were determined 500 μg/mL against *Bacillus cereus* and *Aeromonas hydrophila*).

Therefore, the synthetic thioflavones have a potent antimicrobial activity, and are expected to be used as novel antimicrobial agents.

Synergistic effect on antibacterial activity of violacein by addition of nanostructured silver vanadate

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In this work we report the synergic effect of violacein with silver vanadates nanowires decorated with silver nanoparticles combination. The violacein, a purple pigment produced by *Chromobacterium violaceum*, has shown to have antibacterial activity against methicilin-resistant *Staphylococcus aureus* (MRSA) strains, which makes it a promising antimicrobial compound. This indole-derivative is synthesized from the condensation of two L-tryptophan molecules. Violacein synthesis is known to be induced under aerobic conditions and in response to *quorum sensing*, although physiological function of violacein is not yet clarified. The nanomaterials were obtained through the precipitation reaction of ammonium vanadate and silver nitrate at low temperature (65ºC) followed hydrothermal treatment in different conditions. This hybrid nanomaterial was characterized by XRD, FTIR, RAMAN, TGA, DTA, Diffuse Reflectance UV-VIS, SEM and TEM, and its antibacterial activity was evaluated against strains of *Staphylococcus aureus*. The X-ray diffraction pattern of hybrid nanomaterial was indexed to the $AgVO_3$ phase. The Raman peaks are well defined and easily identified as being related to the beta-$AgVO_3$ crystallized in the monoclinic C2/m phase. By inspecting the TGA and DTA data, we can observe three stages of mass loss from room temperature up to 380ºC. The TEM images showed that the hybrid materials are made of nanowires with micrometric length and diameter ranging from 40 to 100 nm, and they are decorated with silver nanoparticles ranging from 10 to 50 nm. This hybrid silver vanadate nanostructure showed a promising antibacterial activity against strains of S. aureus, where the silver decorated vanadate nanowires act as both support agent for the Ag nanoparticles and Agº source. The silver vanadate nanowires may also have bactericidal action as well thus contributing to increase bactericidal activity of nanostructured system as compared with isolated components. The Minimal Inhibitory Concentrations (MIC) of violacein for *S. aureus* varied between 6.25 and 25 µM, while MICs of nanostructured silver vanadate were above 15 µM. Violacein showed a synergic effect when combined with nanostructured silver vanadate in *S. aureus* stains, including MRSA strains, exhibiting around 10 times lower minimum inhibitory concentration (MIC) than free compounds, respectively. Therefore, this results opens a new perspective in antibiotic therapies against *Staphylococcus aureus* strains, including those that are multi-drug resistant, though further *in vivo* and toxicology studies should be performed.

Keywords: violacein, nanostructured silver vanadate, antibacterial activity, MRSA

International Conference on Antimicrobial Research (ICAR2010)
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Synthesis and Biological Evaluation of Novel Hydroquinones and Benzoquinones as Potential Antimicrobial Agents.

Ibrahim Chaaban[1], El Sayeda M. El Khawass[1], Mona A. Mahran[1], Heba A. Abd El Razik[1]*, Nehad S. El Salamouni[1] and Abeer E. Abdel Wahab[2]

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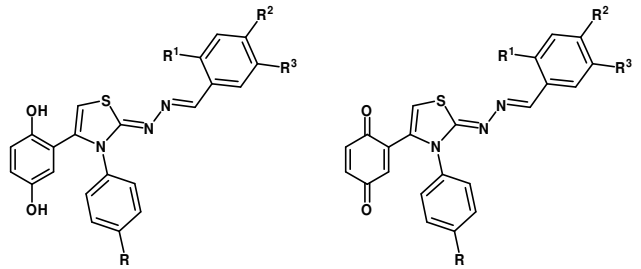
Quinones and hydroquinones are of current interest due to their wide-spectrum bioactivity and chemotherapeutic value. Several quinone and hydroquinone derivatives exhibit antibacterial, antifungal, antiviral and anticancer activities. In addition, several naturally occurring quinones and hydroquinones were reported as potent antimicrobial agents. On the other hand, careful literature survey revealed that thiazole ring systems have occupied a unique position in the design and synthesis of novel biologically active agents with remarkable antimicrobial activities.

In view of the afore-mentioned facts and as a continuation of an on-going program aiming at the synthesis of new quinones and hydroquinones with antimicrobial activities, it was designed to synthesize novel thiazolines connected to hydroquinone or benzoquinone hoping that such combination would result in an increased antimicrobial activity. Twenty four compounds were evaluated for their *in-vitro* antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* as Gram-positive bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative bacteria. They were also evaluated for their *in-vitro* antifungal potential against *Candida albicans.* Some of the newly synthesized compounds were found to possess promising activity.



R = H, CH_3, Cl
R^1= H, OCH_3
R^2, R^3 = H, OH, OCH_3

Synthesis and evaluation of antituberculosis activity of heterocyclic derivatives

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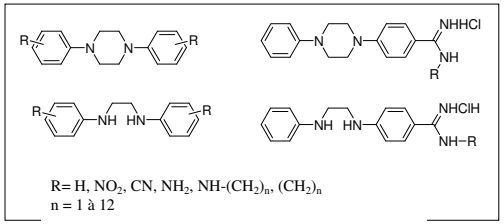
Tuberculosis, caused by *Mycobacterium tuberculosis*, is a major and challenging health problem around the world. At the present time, numerous drugs are available for the treatment, principally combinations of rifampicin, isoniazid, ethambutol, and pyrazinamide. As the actual treatment is long , compliancy is difficult to maintain resulting in increased risk of development of drug resistance. Therefore, there is an urgent need for the discovery of new structural classes of derivatives that would be highly effective against the susceptible and resistant forms of the microbe.

As part of our research efforts on the identification of novel antituberculous drugs, a library of heterocyclic structures has been synthesized using greening chemical protocols[1,2]. Indeed, it is important to conceive experiments enabling to prepare drug candidates in high yields, at low cost, and under benign conditions. For that purpose, we have taken advantage of the rapidity and simplicity of microwave heating to perform chemical transformations. The general structures of these compounds are represented below.



All derivatives of these structures were evaluated *in vitro* against *M.tuberculosis* H37Rv using a luciferase screening assay. We have previously shown that this luminometric method can replace fastidious plating on solid agar for the enumeration of mycobacteria in organ homogenates from infected mice[3] and in macrophage cultures[4].

A compound was considered to be a potential antimycobacterial agent if it resulted in at least 90% reduction in bacterial replication (expressed in relative light units (RLU), 1 RLU = 2 CFU) at a concentration of 1µM.

A few derivatives exerted a promising growth inhibition effect against the TB bacilli. The obtained results revealed that the nature of the subsitution on the benzene ring may have an considerable impact on the antitubercular activity. A second interesting fact observed was that the biological activity seems to be correlated with the length of the carbon chain. Increase of the lipophilic nature of the structure improved the antimycobacterial activity.

Determination of cytoxicity (IC 50) and physico-chemical properties (Lipinski's rule, etc.) of the selected compounds were also included in our screening procedure.

The optimization of the newly identified leads through structure-activity relationship studies is in progress.

Keywords: heterocyclic chemistry, piperazines, Mycobacterium tuberculosis, antimycobacterial activity, lipophilicity

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Synthesis and evaluation of six novel antibacterial fibers disperse dyes based on sulfonamides and their antibacterial activity assessments

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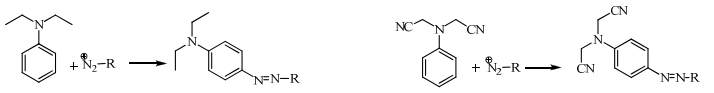
Bacteria are microorganism that grows up in humidity conditions causing many problems. One of the methods to terminate growing of them is the use of antibacterial agents. Sulfonamides are the chemicals that imparting antibacterial properties to a media where they apply. In order to combine the strength of antibacterial activities of sulfonamides and their capability of being diazotized, three novel antibacterial azo desperse dyes based on sulfonamides were synthesized and evaluated. To do this, three sulfonamides viz. amino-N-(4-methyl-2-pyrimidinyl) benzene sulfonamide (sulfadiazine (SFD)), 4-amino-N-(4-dimethyl-2-pyrimidinyl)benzene sulfonamide(sulfamerazine (SFMr)) and 4-amino-N-(4,6-dimethyl-2-pyrimidinyl)benzene sulfonamide (sulfadimidine (SFDd)) were diazotized using HCl and $NaNO_2$ to produce diazonium salts The resultant diazonium salts were then coupled with the coupling component such as N, N-diethyl aniline and N, N-dicyano ethyl aniline to produce the dyes. The synthesized dyes were filtered off, purified and characterized by ^{1}HNMR, FT-IR and Uv-Vis spectrophotometer. The results from spectral data are strongly indicating that the research work was successful. The novel dyes were then applied on cellulosic fabric by conventional method and finally their antibacterial activity assessments were carried out by AATCC 147 test procedure. Bathochromic shift and solvatochromic shift were examined for above-mentioned dyes. The synthesis route for the dyes is shown in the following.



Keywords: disperse dyes; sulfonamides; antibacterial activity

Synthesis and evaluation of three novel antibacterial azo reactive dyes based on sulfonamides and their antibacterial activity assessments

[b]Behnaz Babaii, [a]Javad Mokhtari, [b]Abolfath Akbarzadeh, [b]Bahareh Babaii

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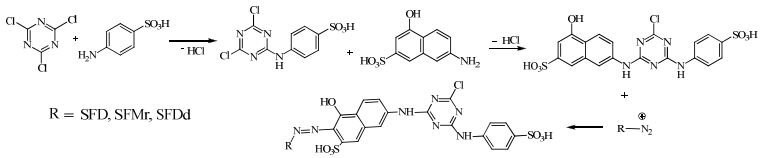
Bacteria are microorganism that grows up in humidity conditions causing many problems. One of the methods to terminate growing of them is the use of antibacterial agents. . In order to combine the strength of antibacterial activities of sulfonamides and their capability of being diazotized, three novel antibacterial azo reactive dyes based on sulfonamides were synthesized and evaluated. To do this, three sulfonamides viz. amino-N-(4-methyl-2-pyrimidinyl) benzene sulfonamide (SFD), 4-amino-N-(4-dimethyl-2-pyrimidinyl)benzene sulfonamide (SFMr) and 4-amino-N-(4,6-dimethyl-2-pyrimidinyl)benzene sulfonamide (SFDd) were diazotized to produce diazonium salts. A coupling component was prepared by the condensation of 6-amino-1-hydroxynaphtalene-3-sulfonic acid (J-acid); 2, 4, 6-trichloro-1, 3, 5-triazine (cyanuric chloride) as a reactive group and 4-aminobenzene sulfonic acid (sulfanilic acid) in two separate steps. The resultant diazonium salts were then coupled with the coupling component to produce the dyes. The synthesized dyes were filtered off, purified and characterized by ^{1}HNMR, FT-IR and Uv-Vis spectrophotometer. The results from spectral data are strongly indicating that the research work was successful. Dyes were then applied on cellulosic fabric by conventional method and their antibacterial activity assessments were carried out by AATCC 147 test procedure. The synthesis route for the dyes is shown in the following.



Keywords: reactive dyes; sulphonamide; sulfanilic acid; antibacterial activity

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The role of enrofloxacin-based-metallocomplexes in the war against bacterial resistance

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Enrofloxacin, a second generation quinolone is, as other fluoroquinolones, a largely prescribed antibiotic in veterinary practice due to its broad spectrum [1]. The quick development of resistance to antibiotics by microorganisms has lead to the need for constant reinvention of these drugs, a context in which the metalloantibiotics can arise as a possible solution [2].

In this study, binary copper(II):enrofloxacin and ternary copper(II):enrofloxacin:phenantroline complexes were analysed, establishing their behaviour in aqueous solution both by dissolution of previously synthesized crystals [3] or by mixture of the aqueous components in stoichiometric proportions. Their antimicrobial activity against *Escherichia coli* ATCC 25922 and BL21(DE3) was tested and, the binary complex showed an efficacy twice that of enrofloxacin alone, justified by the speciation diagrams previously constructed [3]. The ternary complex shows values of minimum inhibitory concentration (MIC) similar to those of the non-complexed quinolone.

MIC values determined for enrofloxacin and its ternary complex on a battery of porin-deficient *E. coli* BL21(DE3) mutants show different OmpF and OmpC dependence which point to different intake pathways and seem to indicate a different translocation route for the antibiotic and the metalloantibiotic.

The hypothesis that microorganisms resistant to pure fluoroquinolones could be sensitive to their metal-complex derivatives has previously been put forward [4] and our data on the microbial susceptibility of Multi Drug Resistant *Staphylococcus aureus* clearly show that, some clinical isolates, exhibit much higher sensitivity to the ternary complex than to the free enrofloxacin.

The overall results are quite encouraging and suggest that the study of the ternary copper complex as a potential new antibacterial agent is worth pursuing.

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Keywords Enrofloxacin, microbial susceptibility, metallocomplex

The Synthesis and antimicrobial effects against some microorganism of 1,3,5-substitutedphenyl formazans

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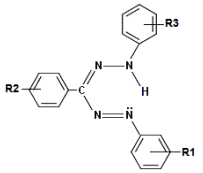
Formazans are colored compounds due to in their structure π-π* transitions of π-electrons. Since Pechman synthesized the first formazans there have been numerous formazans synthesized up to now and their structural features were investigated. Formazans have important medical applications but they are toxic in nature which prevented its routine in health sector. In this study, novel formazans with various substituents on 1,3,5-phenyl rings been synthesized and their structures were elucidated with the use of Elemental Analysis, Mass, ^{1}H-NMR, ^{13}C-NMR, IR, UV-vis spectra (Scheme 1). The goal of this study was to synthesize the compounds for the use of medical purposes. We evaluated antimicrobial effects of formazans against some microorganism. They were evaluated for their antimicrobial activity against *Staphylococus aureus, S. epidermidis, S. saprophyticus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*. Moreover, the antiyeast effects of the formazans are seen on the *Candida kefir, C. glabrata, C. tropicalis, Cryptococcus neofarmans, Saccharomyces cerevisiae*. In the present study, it was generally observed that the formazans were very active against *Candida kefir, C. tropicalis, Cryptococcus neofarmans and Saccharomyces cerevisiae*.



No	(i) Abrev ations	R1	R2	R3
1	NP10	H	H	H
2	NP3	4-OCH_3	4-OCH_3	2-pyridino
3	NP4	4-OCH_3	9-phenantren	2-pyridino
4	NP9	4-OCH_3	4-OCH_3	2-imidazol
5	GA3	4-CH_3	2-OH	2-pyridino
6	GA6	4-CH_3	3-OH	2-pyridino
7	GA9	4-CH_3	4-OH	2-pyridino

Scheme 1. The structure of the formazans derivatives

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The Synthesis of a Series of Quinolone Derivatives and their evaluation against *P. aeruginosa*, *S. Aureus* and *E. coli*.

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In the last number of years we have seen an increase in bacterial resistance to standard drugs, a decrease in hospital hygiene standards and ultimately a rise in hospital acquired infections (HAI's). Ireland currently has the EU's highest rate of *E. coli* infection[1] and the cost of MRSA infection in Irish hospitals has been estimated at €23 million per year.[2] As a result, there is a pressing need for new and more efficient antimicrobial agents.

In the 1960's nalidixic acid, a first generation quinolone, was introduced for treatment of urinary tract infections.[3] Since then numerous quinolone derivatives have been synthesised and one of the most well-known is the fluoroquinolone, ciprofloxacin, **Figure 1**. Quinolones are broad spectrum antibacterials and work by inhibiting bacterial DNA replication by forming complexes with one of two bacterial enzymes; (1) DNA gyrase or (2) DNA topoisomerase IV.[4] The fact that the quinolone molecules show a 1000-fold selectivity for bacterial topoisomerase over the human topoisomerase enzyme makes them very attractive antimicrobial agents.[3]

Current work involves the synthesis and evaluation of a family of quinolone derivatives bearing a series of functional group changes and bioisosteric replacements. We anticipate that these derivatives will show improved antimicrobial activity over existing quinolones. The activity of the new derivatives are evaluated against *P. aeruginosa*, *S. Aureus* and *E. coli*. As many metal complexes are known for their therapeutic applications,[5] including their potential use as antimicrobial agents,[6] the complexation of various metals to these derivatives is also being explored in an effort to further improve activity.

Keywords Quinolone; Antimicrobial, *P. aeruginosa, S. Aureus, E. coli*

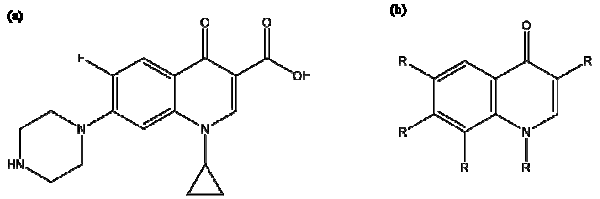

Figure 1. (a) Ciprofloxacin and (b) Generic quinolone structure, R = site of substituent variation

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Towards the rational design of antimicrobial peptides: recent developments in computational tools.

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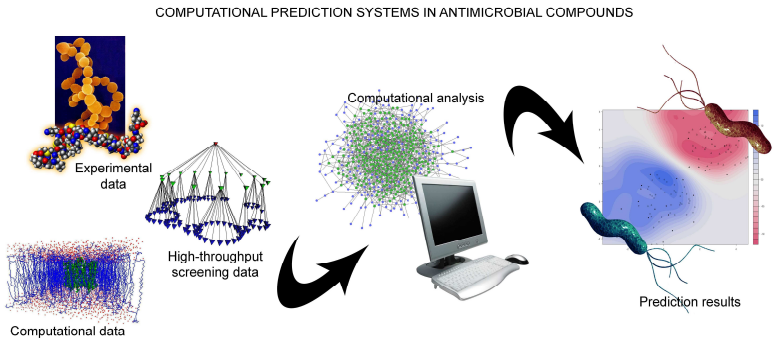
Host defence antimicrobial proteins and peptides (AMPPs) are effectors of the innate immune system and play a vital role in the prevention of bacterial infections. Computational algorithms are useful tools able to predict active fragments in proteins and peptides that can potentially be employed as therapeutic agents to combat bacterial infections.

Some computational methods have been developed in order to find AMPPs with potential application in the pharmacological industry. Among them, we are currently developing a detailed analysis of peptide's physicochemical properties showing that is possible to achieve a high classification score using, essentially, peptide structure and aggregation related parameters. The results show that taking into account only few descriptors we are able to successfully predict active compounds and, moreover, assess its antimicrobial potency in a more limited manner.

However, few efforts have been made in attempting to analyse bactericidal proteins in order to identify the structural determinants involved in their mechanism of action. In this line, we have designed a protein scanning system that is able to perform a fast screening analysis over large protein sets in order to identify potential active peptides (1). The method differs from previously reported predictive algorithms as it is based on high throughput screening experimental results obtained for a synthetic peptide library. In this line, the prediction of antimicrobial stretches in proteins can lead to production of peptides with enhanced antimicrobial activity (2).

In summary, the currently available computational tools can successfully predict antimicrobial sequences in both proteins and peptides. Notwithstanding, more research is needed in order to efficiently estimate their particular antimicrobial potency.

Keywords: antimicrobial peptide; computational analysis.



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Understanding the antibacterial mechanism of ZnO and CuO nanoparticles

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An innovative study aimed at understanding the influence of the particle size of ZnO and CuO (from the microscale down to the nanoscale) on its antibacterial effect has been performed. The antibacterial activity of both ZnO and CuO has been found to be due to a reaction of the metal oxides surface with water. Electron-spin resonance measurements revealed that aqueous suspensions of small nanoparticles of the metal oxides produce increased levels of reactive oxygen species (ROS). However, these metal oxides were found to differ in the nature of the ROS been produced via their surface reaction. Interestingly, a remarkable enhancement of the oxidative stress, beyond the level yielded by the metal oxides themselves, was detected following the antibacterial treatment. Likewise, an exposure of bacteria to the small metal oxides nanoparticles resulted in an increased cellular internalization of the nanoparticles and bacterial cell damage. The examination of the antibacterial effect was performed upon two bacterial species: *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive). The nanocrystalline particles of the metal oxides were synthesized using ultrasonic irradiation, and the particle sizes were controlled using different solvents during the sonication process. Taken as a whole, it is apparent that the unique properties (i.e., small size and corresponding large specific surface area) of small nanometer-scale metal oxides particles impose several effects that govern its antibacterial action. These effects are size dependent and do not exist in the range of microscale particles.

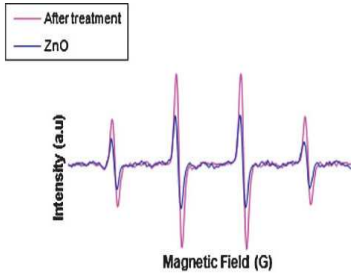

Figure 1. ESR spectra demonstrating changes in hydroxyl radical concentrations upon antibacterial treatment with a water suspension of ZnO np.

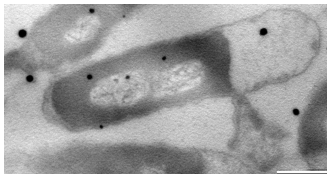

Figure 2.TEM image demonstrating the effect of ZnO nanoparticles on *E. coli* cells.

9. Biocontrol - Biosynthesis of antibiotics – Phages

A class IIa Bacteriocin Isolated from *Leuconostoc pseudomesenteroides:* Structure, Antimicrobial Activity and Organization of the Biosynthetic Gene Cluster

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Many food-grade microorganisms are used to produce a variety of fermented food, among which lactic acid bacteria (LAB) are the most important group. LAB inhibit food spoilage bacteria by producing large amounts of lactic acid and growth-inhibiting peptides termed bacteriocins. Bacteriocins are antimicrobial peptides ribosomally synthesized by bacteria, which are usually active against related species [1]. This has prompted new approaches to inhibit foodborne pathogens, in particular *Listeria* and *Enterococcus*.

Boza is a fermented beverage from the Balkans prepared from cereals, like rice, maize or wheat. Many LAB producing antimicrobial compounds have been isolated from this beverage [2, 3], such as *Leuconostoc*, which produces peptides active against *Listeria ivanovii*, known to be responsible for animal infections and *Listeria monocytogenes*, a major pathogen responsible for serious human disease [4].

We here isolated a bacteriocin-producing bacterium from Boza that we identified as *Leuconostoc pseudomesenteroides* by biochemical and molecular analysis. Purification of the bacteriocin was performed by ammonium sulphate precipitation followed by reversed-phase HPLC. Mass spectrometry analysis indicated a molecular mass of 3930 Da. The primary structure was determined by Edman degradation and ESI-MS/MS, revealing that this antimicrobial peptide is similar to leucocin A/B and belongs to class IIa bacteriocins.
The plasmid-located gene cluster involved in the bacteriocin biosynthesis was identified and analysed. Surrounding the gene encoding the prepeptide, the cluster includes genes encoding an immunity protein, an ATP-dependent transporter and an accessory factor, presumably involved in the bacteriocin export.

Leucocin purified from *Leuconostoc pseudomesenteroides* inhibits the growth of related species, such as *Leuconostoc mesenteroides, Lactobacillus sakei* and *Weissella paramesenteroides*, as well as food pathogens like *Listeria* and *Enterococcus* species. Pathogenic strains, such as *Streptococcus pneumoniae,* are also sensitive to this potent bacteriocin. The minimal inhibitory concentrations determined for the purified bacteriocin are included in the 0.75-2.5 µM range.

It has been shown previously that a σ54-dependent PTS permease of the mannose family is responsible for the sensitivity of *Listeria monocytogenes* and *Enterococcus faecalis* to mesentericin Y105, which is a related class IIa bacteriocin [5]. Antimicrobial assays on mutated σ54 *Listeria* and *Enterococcus* strains are currently performed using the bacteriocin isolated from *L. pseudomesenteroides,* in order to identify if its mode of action also requires σ54.

Keywords antimicrobial peptide; bacteriocin; leucocin; lactic acid bacteria; biosynthesis gene cluster

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A strain of *Trichoderma* as Biological control agent

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Twenty-three strains of *Trichoderma* had been separated by plate dilution method. A strain of *Trichoderma* was screened out because of its excellent antifungal activity, broader antimicrobial spectrum. It also can secrete antimicrobial substances. The antifungal activity of this strain was studied by dual culture tests, Dennis's method (1971) and the disc filter method.

The growth rate of this *Trichoderma*, 4.2 cm/day, was 2 to 3 times faster than the pathogens. Inhibition rate against phytopathogen through dual culture tests was: *Phytophthora capsici* 80.46%, *Botrytis cinerea* 76.65%, *Fusarium oxysporum* f. sp. *niveum* 74.50%, *Fusarium oxysporum* f. sp. *vasinfectum* 73.71%, *Fusarium solani* 72.15%, *Rhizoctonia solani* 66.67 %, *Fusarium graminearum* 63.44%, and *Altenaria solani* 58.18%, respectively.

The inhibition of volatile metabolites from this *Trichoderma* against the above eight pathogens was carried out according to Dennis's method (1971). The inhibition rate was as follow depending on different pathogen: *Altenaria solani* 42.22%, *Botrytis cinerea* 38.52%, *Fusarium graminearum* 34.81%, *Rhizoctonia solani* 22.59%, *Fusarium oxysporum* f. sp. *vasinfectum* 21.48%, *Fusarium solani* 20.37%, *Fusarium oxysporum* f. sp. *niveum* 19.26%, and *Phytophthora capsici* 11.48%.

The inhibition of non-volatile metabolites on the eight pathogens was studied by disc filter method. And the result showed that the non-volatile metabolites from this *Trichoderma* strain possess higher antifungal activity than volatile metabolites did. The highest inhibition rate was 85.93% against *Botrytis cinerea*, secondly was 82.96% against *Altenaria solani*, the worst was 23.70% against *Phytophthora capsici*. The inhibition rate against other five pathogenic fungi was: *Fusarium graminearum* 78.15%, *Rhizoctonia solani* 70.74%, *Fusarium oxysporum* f. sp. *niveum* 52.22%, *Fusarium oxysporum* f. sp. *vasinfectum* 49.26%, and *Fusarium solani* 44.07%.

This *Trichoderma* strain can inhibit pathogenic fungi through different antifungal mechanism in accordance with different pathogen. *Phytophthora capsici* was inhibited by competition and hyperparasitism. For the other seven pathogens, in addition to the competition and hyperparasitism, fungistasis of antimicrobial substances was also an important mechanism. And the inhibition rate of non-volatile substances against each pathogen was about 2 times as much as that of volatile substance, which indicated that the main antimicrobial substance of the *Trichoderma* was non-volatile substance. It is worth while to further study for antimicrobial substances' extraction, separation and purification.

Keywords *Trichoderma*; pathogen; biological control

Activity of terpenoids from *Salvia officinalis* and *Salvia sclarea* against phytopathogenic fungi

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The ethanol extracts of aerial parts of *Salvia officinalis* (common sage) and *S. sclarea* (clary sage) were examined as potential sources on antifungal compounds against plant pathogens. The extract of both species exhibited remarkable antifungal activity against the oomycete *Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni., which is the causal agent of grapevine downy mildew, one of the most important diseases occurring worldwide. The preliminary characterization of the chemical composition of the extracts and the identification of the potentially effective molecules were carried out with chromatographic analyses (CC and GC). The main components of active fractions belong to the terpenes group, as monoterpenes (thujone, camphor, borneol, eucalyptol), sesquiterpenes (caryophyllene, globulol, α-humulene) and diterpenes (sclareol and manool). Other minor constituents were also identified. The antifungal activity of these molecules was evaluated *in vitro* experiments and *in planta*. Chemical composition and biological activity of *S. officinalis* and *S. sclarea* extracts and their major components and potential applications in agriculture will be discussed.

Keywords *Salvia officinalis*; *Salvia sclarea*; sage; antifungal activity; *Plasmopara viticola*; terpenes

Amylolysin, A Lantibiotic Produced by *Bacillus Amyloliquef Aciens* GA1.

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Genome analysis of *Bacillus amyloliquefaciens* GA1 highlights its high potential for antibiotic synthesis. Among them, a peptide antibiotic, named amylolysin was found active against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus.* Several experimental evidences suggest that this peptide belong to the group of class I bacteriocin: the so-called lantibiotics. Presence of the unusual thioether amino acids lanthionine generated through post-translationnal modification has been shown by LC/MS technique. Indeed, the amylolysine prepropetide, encoded by the gene *amyA* contains the highly conserved Type-B lantibiotic motif CTLTXEC. In addition, in *amyM* gene located directly downstream of *amyA*, six conserved motifs involved in the formation of lanthionine, a characteristic feature of lantibiotic, have been identified. Direct interaction of amylolysin with lipid II as well as transmembrane pore formation of target cells supplemented with amylolysin were demonstrated by chromatographic and fluorometric techniques.

Keywords: Class I Bacteriocin; lantibiotics; antibacterial agents;

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Anti-Candida Isolates from Poles

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Isolation and screening of antimycotic substances (AMS) producing microorganisms from Antarctic Penguin Rookery samples, and Arctic sea-water-glacier stream convergence samples was performed. Growth of isolates occurred within the pH range 7.2 –7.5 in the modified Trypticase soya broth (mTSB). The temperature range for growth of the promising isolates was 4 – 22 °C and the growth was observed at 0, 2 and 5% NaCl with optimum growth at 2 % (w/v). As a result of our investigation two *Psychrobacter* species were isolated from the penguin rookery at the Larsemann Hills of East Antarctica which showed moderate antimycotic activity against *C albicans* NCIM 3471. Two Arctic isolates were grown at 15°C for 48 hours and the cell free supernatants (CFS) showed antimicrobial activity against several different strains of multidrug-resistant *Candida albicans*. The concentrated cell-free supernatants were able to demonstrate increased inhibition against three different *Candida albicans* strains in a cut-well agar assay. The concentrate mildly inhibited *C .krusei* NCIM 3129.

Two of the AMS producers belonged to species *Carnobacterium maltaromaticum and Yersinia indermedia,* based on 16S rDNA gene sequences and fatty acid compositions analyses respectively. 16S rDNA gene sequence analysis showed 99.8% similarity between strain AGM 111 and *Carnobacterium maltaromaticum*.

Keywords Antarctic;antimicrobial substance; antifungal substances; CFS; candidiasis.

Antibiotic activity of Bacillus

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A screening of several *Bacillus* species showed that they produce and export a number of low molecular weight compounds as was determined by MALDI-TOF mass spectrometry both in culture supernatants and intact cells. Some of these substances, lipopeptides such as surfactins, iturins (1043-1095 m/z) and fengycins (1463-1505 m/z) are antibiotics. Smaller molecules (500-843 m/z), in principle identified as lipids, correspond to cell wall components since were found only in intact cells. Other (500-700 m/z), are secreted products because were detected both in intact cells and in the culture medium. A *Bacillus megaterium* strain releases a mixture of these compounds displaying antibiotic activity against both Gram positive and Gram negative bacteria. Species highly sensitive to the mixture are from the genus *Bacillus* such *B. lentus, B. polymixa, B. circulans*, *B. cereus* or *B. subtilis*. Dynamic laser light scattering by fresh *B. megaterium* supernatants reveals particles having mean diameter consistent with vesicles or micelles. These structures are apparently assembled spontaneously in a mineral culture medium (MM) made up from salts, nitrogen and carbon sources. MALDI-TOF mass spectrometry of both raw and dialysed supernatants against water or MM, are qualitatively similar displaying the whole set of substances. However, striking differences among peak intensities are observed after dialysis against water or MM. Those corresponding to 500-700 m/z are not affected, 1043-1095 m/z peaks are severely removed while 1450-1505 m/z peaks remain relatively unchanged. If after dialysis the suspension is centrifuged, the sole change occurs at the intensities of 500-700 m/z peaks, which are strongly diminished. This behavior suggests a distinct contribution of these substances in assembling particles detected by laser light scattering. Thus, apparently the larger particles are organized around the 500-700 m/z substances. These are not dialysed but are centrifuged. Fengycins (1463-1505 m/z) are assembled in particles which may or not contain 500-700 m/z compounds. They are neither dialysed nor centrifuged. The 1043-1095 m/z substances appear to be dialysed, an indication that they are not included in particles. These results suggest that the antibiotic activity exhibited by this *Bacillus megaterium* strain is related to free lipopeptides (1043-1095 m/z) and/or to particles (vesicles, micelles) which may be formed by fengycins and low molecular weight lipids. Since other *Bacillus* species export a similar set of substances, these observations may also be used to explain their antibiotic activity.

Antifungal lactic acid bacteria with potential to enhance dairy products conservation

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Fungi and yeasts are most common spoilage organisms of food. In addition to economic losses, the potential production of toxins is of particular health concern. In the recent years, increasing interest has been shown in biopreservation over chemical methods due to consumer demand. Among natural biological antagonists, lactic acid bacteria (LAB) have a long history of use in food. The use of LAB to control mycotoxinogenic fungal could represent an efficient strategy, since it has been reported that these bacteria have strong antimicrobial properties.

The aim of the present study is to characterize the ability of LAB isolated from raw milk to repress the growth of fungi. Several isolates were tested against *Penicillium roqueforti, Geotrichum candidum*, *Mucor plumbeus*, *Cladosporium*, *Kluyveromyces lactis* and *Debaromyces hansenii* by overlay method. The potent antifungal isolates display a wide range of fungi inhibition and were identified by phenotypic characters as lactobacilli. Production of the antifungal compounds started at the end of the exponential growth phase and reached a maximum after 48 h of culture.The chemical nature of these substances is under characterization.

Keywords: Lactic acid bacteria, antifungal substances.

Antifungal lactic acid bacteria with potential to prolong shelf-life of low salt bread

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Mean daily salt intakes of populations in developed countries are well in excess of dietary needs (ca. 3-4 g salt/day). Hypertension, a causal factor for cardiovascular diseases, was shown to be caused by increased amounts of sodium, which is mainly applied as sodium chloride (table salt). Up to 35 % of the daily salt intake is contributed by cereal products, in particular bread. Hence, low-salt bread is one of the most efficient ways to decrease the daily salt intake. The technological process of bread baking as well as some of the final quality characteristics of bread, in particular shelf-life is influenced by salt reduction. Chemical preservatives, e.g. calcium propionate (CP) are commonly used as antifungal agents. Alternatively, sourdough can be used to retard mould growth. This work addresses the feasibility of salt reduction in wheat bread from 2.0 %* (standard) to 1.0 % (sodium-reduced), 0.5 % (low-sodium) and 0.0 % (sodium-free) from shelf-life perspective. The results were compared to those obtained using 0.5 %* of CP and 20 %* of sourdough fermented by the antifungal strain *L. amylovorus* DSM 19280. The antifungal "*in situ*" tests were performed under bakery environmental conditions as well as using challenge tests against *F. culmorum, A. niger* and *P. expansum*. Mould growth on the bread slices was observed throughout 14 days of storage. For the environmental trials, a shelf-life of about 5 days was obtained for standard bread (2.0 % salt) while breads elaborated with lower salt concentration were spoiled after 3 days. Sourdough addition prolongs the shelf-life at least up to 12 days and the addition of 0.5 % CP prolonged the shelf-life 10-12 days compared to the respective controls without any significant differences regarding the salt levels. Concerning the fungal challenge tests, the spoilage was influenced, with different extent, by both salt level and the fungi tested. Generally, similar antifungal performance was observed in sourdough breads and CP breads when tested against the indicator moulds. The findings of this study indicate that addition of sourdough fermented with the antifungal *L. amylovorus* DSM 19280 can replace CP addition needed to assure the safety of low-salt bread.

* Baker's percentages

Keywords Lactic acid bacteria, sourdough, low salt bread, shelf-life, antifungal activity

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Antimicrobial activity of moderate halophilic actinomycetes strains isolated from saline soil in region of Bejaia

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A total of 156 different isolates of moderate halophilic actinomycetes were isolated from saline soil on Starch Casein Agar medium collected from Bejaia. All the 156 actinomycetes isolates exhibited antibacterial activity against human bacterial pathogens: *Staphylococcus aureus, Micrococcus luteus* and *Bacillus subtilis.*

Based on screening results, four potential actinomycetes were selected and tested for their antifungal activity against three pathogenic fungi: *Aspergillus niger, Fusarium polyferatum, Mucor ramanianus* and against the yeast *Candida albicans.* These isolates showed remarkable antibacterial and antifungal activity.

The taxonomical properties of the strains were examined. The primary identification of the 4 isolates was based on spore morphology and cell wall chemo-type. 3 of these strains belonging to *Streptomyces* genus, however, the other isolate named S6 is affiliated to the genus *Streptoverticillium.*

According to the physiological study, the tested strains could tolerate NaCl concentration of 75g/l.

Production of overall antifungal and antibacterial activities was checked on Williams modified medium (7,5% NaCl).

The antibiotics were extracted with different solvant and detected by bioautography on silica gel plates using *Bacillus subtilis* as the test organism.

The UV–visible spectrum and the chromogenic reactions of this active compound suggested a non-polyenic nature and probably polyether structure. Investigation of these molecules is now in progress.

Key words: Halophilic actinomycetes, Saline soil, *Streptoverticillium*, Antibacterial activity, Antibiotics, Bioactive molecules.

Application of novel alkalotolerant Actinomycete spp as biocontrol agents against fungal plant pathogens and as plant growth promoters

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Actinomycetes are well known for their ability to produce several biologically active compounds, which may have antibacterial and antifungal properties. The increased incidence of fungal infections, especially dangerous hospital acquired infections and infections in immunocompromised patients, has accentuated the need for new, safe and more effective antifungal treatments. Furthermore, in the field of agriculture, public pressure to reduce the use of chemical fungicides has increased. Concerns have been raised about both, the environmental impact and the potential health hazards related to the use of these chemicals.

Biological control approaches an interesting substitute to synthetic fungicides. The alkalotolerant actinomycete strain A-03-1160 exhibiting antagonistic effect against several pathogenic fungi. In preliminary results in shake flasks, it was found that the actinomycete culture caused complete degradation of various fungi such as *Mucor, Aspergillus niger, Aspergillus oryzae, Trichoderma reesei, Alternaria solani, Fusarium moniliforme, Curvularia fallax, Curvularia lunata, Claviceps purpurea, Helmintosporium*, etc. indicating that the organism could be used as a potential bio-control agent. Coating the seeds of *Cicer arietinum* with the spores provided protection against fungal contamination during germination.

The antifungal activity was found to be largely extracellular. The crude filtrate and the mycelial extract were devoid of 1, 3 glucanase and chitinase activities. The culture filtrates and the mycelia were extracted with ethyl acetate. The residue after extraction showed antifungal activity when tested against several fungi, including plant pathogens. The MIC of the purified compound for Aspergillus sp was determined to be 1µg/ml which is well within the range allowed for antifungal compounds used.

Keywords: Actinomycetes; Antifungal; Plant pathogens

Bacteriophages to challenge Salmonella, *Serratia marcescens* and *Bacillus subtilis* in foods

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The attention in using bacteriophages as biocontrol agents in foodstuffs is recently increasing. Phages seem to be a more natural alternative to traditional approaches for food sanitation and preservation. They could also be considered in the hurdle technologies application in combination with different preservation measures.

In this study we considered the influence of phages addition on the fate of pathogenic and spoilage microorganisms in different foods. In particular, we tested the strains *Salmonella enterica* serovar Thyphimurium LT2, *Serratia marcescens* DSMZ14187, *Bacillus subtilis* DSMZ5547, in combination with their phages P22, ke49 and phi29, respectively. Furthermore, *Salmonella* Thyphimurium (12 isolates), *Salmonella* Enteritidis (1 strain), *Salmonella* Derby (1 strain) and *Salmonella* New Port (1 strain), all from different food-matrices, were used in this study.

Phages were applied to: liquid eggs, energy drinks, whole and skimmed milk, apple juice, chicken breast and chicken mince all spiked with their respective hosts, whose growth was monitored for 24 and 48 h at 4 °C. The experiments were carried out using two inoculation levels of each bacterial strain (10^4 and 10 CFU/g), and to both low (10^2) and high (10^6) multiplicity of infection. Appreciable host inactivation, generally in the order of 2 log cycles, were achieved compared to phage-free controls in all food matrices when 10^4 UFC/g host inoculum was performed. All the wild *Salmonellae* were assayed against phage P22 and proved to be sensitive towards the phage except *Salmonella* New Port strain.

PCR-dependent fingerprinting of the *Salmonella* strains from all the different sources only allowed differentiation of Thyphimurium from non-Thyphimurium strains. Additional challenge experiments were carried out by spiking liquid-eggs, chicken breast and chicken mince with four-isolates mixes of *Salmonella* Thyphimurium (at concentration of about 10^4 UFC/g) along with a their relative phage P22. The results showed a reduction of 2-3 log cycles after 48 h at 4 °C depending on both mix of strains and the specific food.

Overall, the results indicate that phages may be useful in the control of foodborne pathogens and spoilage microorganisms. The food matrices considered, the liquid more than the solid, do not seem to affect the phage ability of infection compared to similar tests performed *in vitro*. Since the food matrices could have a lower level of bacterial contamination than the experimentally contaminated foods, the use of the bacteriophages as an efficient control in the foodstuffs is promising.

Keywords: bacteriophage biocontrol; *Salmonella* Thyphimurium

BACTIBASE: database mining for bacteriocin discovery

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Bacteriocins are very diverse group of antimicrobial peptides produced by a wide range of bacteria and known for their inhibitory activity against various human and animal pathogens. Although many bacteriocins are now well characterized, much information is still missing or is unavailable to potential users. The assembly of such information in one central resource such as a database would therefore be of great benefit to the exploitation of these bioactive molecules in the present context of increasing antibiotic resistance and natural bio-preservation need. Thus, we developed BACTIBASE, an open-access database dedicated to bacteriocins. The purpose of the database is to serve the research community by organizing information relevant to all types of bacteriocins from all groups of Bacteria. BACTIBASE database brings together physicochemical, structural, taxonomic, spectrum of activity and literature data for bacteriocins produced by both Gram-positive and Gram-negative bacteria. The provided features should make BACTIBASE a useful tool in food preservation or food safety applications and could have implications for the development of new drugs for medical use. BACTIBASE is freely available at http://bactibase.pfba-lab-tun.org.

Keywords bacteriocin; database; BACTIBASE; molecular sequence; data mining

Biocontrol of *Salmonella* Enteritidis in chicken skin

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The presence of pathogenic bacteria such as *Salmonella* and *Campylobacter* in poultry is a public health problem and the cause of large economic losses in both developed and developing countries. The procedures of decontamination by physical and chemical agents used in meat processing industry are an alternative to reduce or eliminate micro-organisms from carcasses. However, the use of these methods may alter the sensory characteristics of meat and can give rise to toxic compounds and lead to selection of resistant micro-organisms. Due to these factors, there are some difficulties in international trade in poultry meat, particularly between the United States and the European Union, due to the use of antimicrobial decontamination of poultry carcasses. Bacteriophages are specific viruses of bacteria that have been studied with the aim of controlling pathogenic microorganisms and spoilage in the food industry, and examples of such applications have been published mainly in the control of *Salmonella* sp[1], *Campylobacter* sp[2], *E. coli*[3], *L. monocytogenes*[4], *C. muytjensii*[5] and *S. aureus*[6]. Seeking alternative ways to control microorganisms in food and with a view to increasing consumer demand for healthier products, this study aimed to evaluate the activity of bacteriophages to reduce *Salmonella* Enteritidis in chicken skin by comparing the efficiency of this treatment method of chemical decontamination. We used randomized block design with seven treatments arranged in 10 blocks and three replicates per block. Each block was composed of fragments of skin from the chest area of 4 cm^2. The fragments were decontaminated by irradiation to 10 kGy for 2 h and then inoculated with10^5 UFC.cm^{-2} of *Salmonella* Enteritidis (ATCC 13076). For comparison with the chemical decontamination, fragments of skin were immersed in polihexametileno biguanide hydrochloride (PHMB) to 769.08 mg.L^{-1} for 15 min; solution of lactic acid 2 % (v/v) for 90 s; solution of sodium dichloroisocyanurate to 200.5 mg.L^{-1} for 10 min; the sanitizing solution based on peracetic acid to 112.3 mg.L^{-1} for 10 min. For the tests using bacteriophage fragments of skin were immersed in a suspension composed of a mixture of five bacteriophages in equal proportions with concentration 9.46 log PFU.mL^{-1} for 30 min. The control treatment was done using sterile water. Decontamination was carrying out at 4 °C and skin fragments were subjected to *Salmonella* count agar XLT_4 after the time of contact with the antimicrobial agents. The result of the count of *Salmonella* in chicken skin fragments in the control treatment was 5.5 log UFC.cm^{-2}. We observed a reduction of 0,2 log UFC.cm^{-2} the count of *Salmonella* from samples immersed in sterile water for 30 min as a consequence of leaching due to the methodology adopted. Treatments with solution of sodium dichloroisocyanurate, peracetic acid, lactic acid solution and suspension of bacteriophages reduced the Salmonella counts on average between 0,81 a 0.92 log UFC.cm^{-2}, did not differ significantly by *Duncan's* test at a significance level of 5 %. The treatment with PHMB solution showed a reduction of 2.7 log UFC.cm^{-2} in count of *Salmonella*. The use of antimicrobial chemicals to reduce *Salmonella* in poultry is being widely researched observed reduction values between 0.5 to 3 cycles logarithms and depend on the type and concentration of an antimicrobial, such as sample, initial microbial load, contact time and application temperature. In studies with bacteriophages, other authors[7] have observed decreased 2 - 3 log UFC.cm^{-2} of *Salmonella* in raw and cooked meat stored at 5 °C, and reduction values greater than 5.9 log UFC.cm^{-2} in samples stored at 24 °C, after application of bacteriophages for values of MOI (multiplicity of infection) of 10^4 at higher contact time (24 h) when used in this study. Reductions of 90 % in the prevalence of Salmonella in chicken carcasses were observed in other studies[8] after application of a spray of bacteriophages in the concentration of 10^9 PFU.mL^{-1}. Despite the low count reduction of Salmonella, in comparison with other studies of application of bacteriophages, the results indicate that treatment with a mixture of five bacteriophages showed activity equal to other decontamination methods studied and commonly used in the poultry industry, so can be a complementary technology for control of pathogenic micro-organism.

Keywords: Bacteriophages, biocontrol, *Salmonella* spp.

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Cell growth control by zymocin, a tRNase ribotoxin from yeast

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Endoribonuclease toxins secreted from prokaryotic and eukaryotic microorganisms constitute a sophisticated strategy that ensures survival of their producers against other microbial competitors. Often, these ribotoxins target tRNAs [1] and tRNA cleavage and tRNA depletion by zymocin, a trimeric ribotoxin complex secreted from dairy yeast *Kluyveromyces lactis*, has recently been shown to cause cell death of baker's yeast *Saccharomyces cerevisiae* [2]. The tRNA attack by zymocin is directed towards tRNA anticodons that possess specific nucleobase modifications at their wobble position [3]. Intriguingly, the pathways required for generating these wobble modifications have been conserved between lower and higher eukaryotes [4]. Therefore, our idea was to take the basic biology of tRNase ribotoxins and apply this to a clinically relevant setting using HeLa model tumour cells whose proliferation heavily relies on tRNA functioning for protein synthesis. In order to assess the effect of zymocin, we monitored HeLa cell proliferation in response to conditional expression of the tRNase ribotoxin from yeast. Our pilot findings indicate that the tRNase does not solely inhibit yeast growth but also affects the viability of higher eukaryotic cells. Hence, our data suggest that microbial tRNase toxins may be invoked as novel anti-proliferative factors for biomedical intervention schemes.

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Keywords: *K. lactis*; zymocin; ribotoxin; *S. cerevisiae*; anticodon tRNase; tRNA modification

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Characterization of Antibacterial Substance-Producino *Baciilus subtilis* IS-1 Isolated from korean Traditional Food *Jeot-kal*.

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A bacterium which has high enzymatic activities such as amylase, cellulose and protease was isolated from Korean traditional food, *Jeot-Kal*. The isolated bacterium was identified to bacillus subtilis IS-1 by the test of morphological and biochemical properties according to Bergey's Manual of Systematic Bacteriology and API 50 CHL kit, and by the 16S r DNA sequence.

The isolated *B. subtilis* IS-1 had a potent antibacterial activity against fish pathogenic bacteria or food pathogenic bacteria. *B.subtilia* IS-1 is endospore forming cell and contained flagella and abundant viscous material at the out layer of cell wall. It was rod type bacterium (0.5~0.8 x 3~5um) having biochemical characteristics such as pram staining(+), catalase(+), oxidase(-) and hydrolysis of esculin(+). The opimum temperature and pH of the growth of the *B. subilis* IS-1 was 35°C and Ph 7, respectively. The antibacterial activity was able to inhibit the growth of pathogenic bacteria including *Vibrio parahaemolyticus, V. harveyii, V. mimicus, V. vulnificus, V. alginolytics, V. salmonicid*, *Edwardsiella tarda*. The opimal culture time for antibacterial activities of the bacterium were shown to be in the range of 12 – 36hr.

Characterization of *Lactobacillus plantarum* TENSIA antimicrobial properties

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Background: the food-borne illnesses are one of the most widespread health problems. The most common bacteria that cause food-borne infections are *Campylobacter*, *Salmonella* and *Listeria*. Some studies show inhibition of these enteric pathogens by indigenous lactobacilli of human origin and probiotic *Lactobacillus* strains.

Objective: the aim was to investigate the antimicrobial properties of *Lactobacillus plantarum* TENSIA DSM 21380 against different enteropathogens.

Material and methods: *Lactobacillus plantarum* TENSIA was studied for antimicrobial properties in the different experimental conditions. Streak line method (Schröder 1975; Annuk et al. 2003) was used for screening antimicrobial properties of TENSIA metabolites and agar spot diffusion method (Jimenez-Diaz et al. 1993, Todorov et al. 2004) was used for detection of the presence of crude bacteriocin.

Target pathogens were *Listeria monocytogenes* (2 strains), *Salmonella* (2 strains), *Campylobacter jejuni* (3 strains), *Shigella sonnei*, *Esherichia coli*, *Enterobacter sakazakii* and *Yersenia enterocolitica*.

Results*:* according to streak line procedure *L. plantarum* TENSIA expressed strongest inhibition against *E. coli* (29.8±3.7 mm); against *S. sonnei, Salmonella*, *E.sakazakii* and *Listeria* the inhibition was somewhat weaker. The antagonistic activity against *Y. enterocolitica* and *C. jejuni* was low (13.5±1.7 and 12.9±5.2 mm accordingly).

Antagonistic activity of crude bacteriocin was stronger in acidic environment, probably because of synergistic action of metabolites, but incubation temperature had no impact to bacteriocin activity.

Supernatant of TENSIA grown at 15°C in MRS media had antagonistic activity against listeria already on the 10th incubation day and it remained until the end of incubation period (30th day). Supernatant of TENSIA grown in milk had antagonistic activity against listeria from the 20th incubation day and the activity also remained until the 30th day, but it was however weaker compared to antimicrobial activity of supernatant from MRS ($p<0.01$).

Conclusions: *Lactobacillus plantarum* TENSIA expresses strong antimicrobial activity against different enteric pathogens. In tested experimental conditions the antimicrobial activity of *L. plantarum* TENSIA is caused by organic acids but probably also by production of bacteriocin as well. The putative bacteriocin needs further characterisation.

Keywords: Lactobacillus plantarum, antimicrobial activity, bacteriocin.

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Characterization of microencapsulates of bacteriophages

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Encapsulation, or more appropriate, the imobilization in particles, is a process in which cells are retained inside semi-permeable polimeric spheres, with the cells uniformly distributed within. The process consists of the mixture of the cells with a prepolimeric solution, applying a force that separates the mixture cell/polymer in ,particles, generally spherical, and followed by material solidification. The polymers which are commonly used include the alginate, gellan gum, carragena, agarose, polyurethane, polyacrylamide and methacrylate. The technique of micro-spheres formation can include the addition of calcium chloride, zinc chloride, different oils, surfactant agents among other. Depending on the polymer and the formation technique used, the particles may vary in size, from very little (approximately 2 to 10 µm of diameter) to very big (approximately 3 mm). The type of polymer and the particle size influence in the cellular activity, retention, nutrients diffusion and particle stability. The use of encapsuled cells has several advangates regarding the formulation done with free cells, including the protecion of the biotic stress, the protection of the abiotic stress such as the inhibitory effect of the toxic compounds. Thus, when fagotherapy is used the encapsuling can improve the distribution of the micro-organism allowing its adequate dosage in different applications. This present study aimed to evaluate different bacteriophages encapsuling processes for the use in the *Salmonellla* Enteretidis control, evaluting the morphology of different types microencapsuled of bacteriophages obtained by optical microscopy. The bacteriophages Lamp40, Lamp41 and Lamp43 were obtained from the bank of phagos from the Laboratory of Pathogens Microbiology of Food and Hydric Origin – LAMPOAH, at the Department of Food Technology, from the Federal University of Viçosa, State of Minas Gerais, Brazil. The respective bacterophages were grown for 18h until a concentration of 10^{10} PFU.mL^{-1} was obtained, followed by a preparation of bacterophages suspension. For treatment 1, the bacterophages were suspensed in solution containing Sodium Alginate (Spectrum, Gardena, Ca) at 1.5 %, surfactant Span-85 (Sigma, St. Louis, MO), which was homogenized and through dripping calcium chloride at 0.5% was added; for treatment 2 the same process was done, however, Tween 20 was added (commercial brand Cinética). For treament 3 the suspension was done in solution containing only the sodium alginate (Spectrum, Gardena, Ca) at 10 %, adding through dripping calcium chloride at 2.5%. The obtained pellets were stored at 8 ºC ± 2 ºC, for 72 hour and then the morphological analyzis was done for each treatment. The microencapsuled evaluation with the addition of the bacterophages obtained through optical microscopy, allowed the observation of their surfaces topography. The observation of the microencapsuled surfaces in treatment 1 and 2 presented themselves in a more heterogenic pattern, of more irregular forms, once re-entries in the format of irregular bubbles in higher or lower quantity were verified, depending on the used surfactant. Microencapsuled 3 was the one that presented the higher homogeneity, with formation of few and small superficial bubbles, very distant and inferior to those presented in the other microencapsuled bacterophages. This homogeneity could be associated to the calcium alginate polimeter formation without the surfactant addition. According to the obtained results and considering the advances that occurred in the microencapsuling field, one can conclude that there is a necessity for further researches regarding the process, since the bacterophages microencapsuling present itself as a quality control tool in the industry in general, once it can have an important role in the food safety.

Keywords: microencapsuling, bacterophages, surfactants, *Salmonella* Enteretidis.

Collimonas: Fungus-eating soil bacteria with great potential for discovery of novel antimycotics

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The genus *Collimonas* consists of soil-inhabiting bacteria that fall within the family of *Oxalobacteraceae*, subclass Betaproteobacteria. So far, three species have been described: *C. fungivorans*, *C. pratensis*, *and C. arenae*. *Collimonas* bacteria are known for the ability to feed on living fungal hyphae. Production of antifungal secondary metabolites may contribute to this feeding behaviour. Several *Collimonas* strains have been reported to inhibit fungi in in-vitro inhibition assays. The range of fungi affected by antifungal activities of *Collimonas* bacteria is species- or strain-dependent pointing at production of different antifungal metabolites by different *Collimonas* strains. Therefore *Collimonas* is an interesting genus of bacteria to mine for genes and compounds involved in antifungal activity.

As an example, the antifungal activity of *Collimonas* bacteria towards the ascomycete *Aspergillus niger* will be presented in more detail. Several *Collimonas fungivorans* strains are antagonistic against *A. niger*. Among them is *C. fungivorans* Ter331, a strain of which the genome has been sequenced. The screening for random plasposon mutants of strain Ter331 with abolished antifungal activity led to the identification of a gene cluster involved in the production of a novel bioactive compound. The comparison of the antifungal activity of strain Ter331 and the above mentioned mutants showed that the same compound is likely also responsible for the inhibition of other *Aspergillus* and *Penicillium* spp. that are known as food spoiling fungi.

Keywords *Collimonas*; antifungal; *A. niger*

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Combined treatments improve the efficacy of biocides and enterocin AS-48 against *Salmonella enterica* cells.

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Introduction. *Salmonella enterica* is a common cause of human foodborne enteric diseases. Disinfection of food contact surfaces and good hygienic practices are key step for prevention of transmission of this (and other) human pathogen through the food chain. Biocides are commonly used as sanitizers in the food industry. Bacteria challenged with biocides may acquire or develop biocide resistance mechanisms. Failure of biocide treatments due to microbial resistance is a threat when food spoilage or human pathogenic bacteria are involved in the resistance. The application of biocides in combination with other antimicrobial substances is an attractive approach in order to avoid proliferation of biocide-resistant strains and to increase the efficacy of biocides. Among the antimicrobial substances of interest are polycationic compounds, chitosan, chemical preservatives, antibiotics, and bacteriocins.

Polymyxin is a membrane-active compound that shows synergistic effects with other hydrophobic antibiotics. A recent study showed that polymyxin B in combination with the bacteriocin nisin showed synergistic effect against Gram-positive and Gram-negative bacteria. The bacteriocin enterocin AS-48 is a cyclic antimicrobial peptide of great interest for biopreservation in the food industry. The present study aims at improving the efficacy of biocides against *S. enterica* in combination with enterocin AS-48 and other antimicrobials.

Material and methods. Six strains of *S., enterica* isolated from outbreaks of salmonellosis were challenged with commonly used biocides, alone or in combination with enterocin AS-48. Chitosan, polyethylenimine, 2-nitropropanol and polymyxin B were also tested alone or in combination with enterocin AS-48, biocides, or both. Assays were carried out in 96-well microplates, and bacterial growth was determined with a microplate reader. The minimum inhibitory concentrations (MICs) were determined after 24 h incubation at 37ºC.

Results and conclusions. All strains were highly sensitive to the biocides hexadecylpyridinium (MIC 0.005%), benzalconium chloride, cetrimide and hexachlorophene (MIC 0.025%) and triclosan (MIC 0.00025%, except one strain with MIC > 0.25%). By contrast, most strains showed a high tolerance to chlorhexidine (MIC ≥0.25%). Synergistic effects of biocides added in combination with enterocin AS-48 in a concentration range of 50 to 250 µg/ml were seldom observed. The single addition of chitosan, polyethylenimine, 2-nitropropanol and polymyxin B resulted in growth inhibition only for 2-nitropropanol and polymyxin B. Combinations of polymyxin B (at concentrations above 2 µg/ml) in combination with enterocin AS-48 (50 µg/ml) resulted in complete growth inhibition of *S. enterica*. Combinations of polymyxin B (2 µg/ml) and enterocin AS-48 (25 or 50 µg/ml) increased the efficacy of biocides (especially for chlorhexidine, hexadecylpiridinium, triclosan and cetrimide) against *S. enterica*. In some cases, the biocide minimum inhibitory concentration was reduced by ten fold compared to the single biocide treatment. These results suggest novel applications of the combination polymyxin B/enterocin AS-48 and biocides against foodborne pathogenic bacteria such as *S. enterica.*

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Keywords: biocides; Salmonella; enterocin.

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Comparison of Anti-Listerial Effect Spectrum of Bacteriocins

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Listeria spp. is one of the important responsibilities of food-borne outbreaks. Therefore, studies of *Listeria* spp. and *Listeria* spp. inactivation treatments have gained great importance and intensity in worldwide. *L. monocytogenes* is the most important and most studied were made on *Listeria* spp. strain. *L. monocytogenes* is a gram positive pathogen and it can be found in a wide variety such as raw and processed animal or plant foods. It can be adapt and grow a wide range of environmental conditions such as low temperature (2-4 ^{o}C), acidic pH and high salt concentration. Due to fatal listeriosis, it was definitely not allowed finding in processed food. In recent years, minimally processed and ready to eat foods are frequently consumed, increased the risk of food-borne listeriosis. As a protection strategy, bacteriocins or bacteriocins producing lactic acid bacteria has created a remarkable effect about food-borne pathogen *Listeria monocytogenes* biocontrol. Many studies on this issue are emphasized, using of nisin, pediocin PA-1/AcH and enterocin A high anti-listerial activity. However, effect of nisin is usually against Gram negative. For high anti-listerial activities with using nisin, to be necessary low pH, NaCl concentration, EDTA and other protective factors such as pulsed electric field and high hydrostatic pressure, different processing method with combined. According to recent studies, the Class IIa bacteriocins provide the most effective anti-listerial effect, including pediocin PA-1/Ach, enterocin A, sakacin P and curvacin A. In this manner, using of Class IIa bacteriocins in variety food such as particularly nisin wasn't very active in meat products, with their strong anti-listerial efficiency, suggested that important potential as biopreservative.

In this review, anti-listerial effect spectrums of bacteriocins produced by different groups of microorganism were compared and potential use of these bacteriocins as biopreservative in food was evaluated.

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Diversity of non-ribosomal lipopeptides in *Bacillus pumilus* from different sources

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Bacillus sp. have been extensively studied for the production of a variety of non-ribosomal lipopeptide (NRL) compounds with antibacterial and antifungal activity. These compounds have a broad spectrum of biological activities and consequently several potential applications in medicine, agricultural and environmental fields. In addition, clinical trials on humans and animals have shown that they have a low toxicity. Surfactin (that encompasses structural isoforms as lichenysins), fengycin (comprising plipastatin variant) and iturin (where iturin A and mycosubtilin were described as variants) families are representative of NRL produced by different species of *Bacillus*. Few strains of *Bacillus pumilus* from private and public collections have been listed as lipopeptides producers. The significance of this specie is also highlighted by the fact that they have been used as probiotics, in animal feed supplements and in aquaculture applications.

The aim of this work is to identify putative *B. pumilus* lipopeptides' producer using a PCR screening strategy. This work is of considerable interest since classical methods seem to be too laborious and hence not applicable for the purpose of rapid screening. A collection of 32 isolates recovered from: i) medicines samples (n=12), ii) cosmetic products (n=5) and iii) food samples (n=7) detected as contaminants, iv) gastropods (n=3) belonging to animal flora and v) plant samples (n=4). *B. pumilus* ATCC 14884 was also included in the study. Identification was performed via biochemical and phenotypic characteristics by Gram stain and BBL Crystal Gram Positive ID Kit and sequencing of 16SrDNA. PCR analyses were performed for the detection of the lipopeptides biosynthetic genes for surfactin (*srf*), liquenisin A(*lchAA*), B (*lchAB*) and C (*lchAC*), iturin A (*ituD*), mycosubtilin (m*yc*), fengycin (*fen*) and plipastatin (*pps*), using primers and conditions described for other *Bacillus* species. Amplicons sequencing was performed for identity confirmation. Hemolytic activity was evaluated using blood agar plates.

The surfactin gene was the most frequent NRL (30/32, 93,75%), occurring together with mycosubtilin gene in 40,6% (13/32) of the isolates or with mycosubtilin and fengycin genes in 18,8% (6/32), with fengycin gene in 12,5% (4/32), with mycosubtilin, fengycin and liquenisin A in 9,4% (3/32), and with mycosubtilin and liquenisin A in 3,1% (1/32). Plipastatin gene was present in 1 isolate, also positive to iturin family. Toward iturin A, liquenisin B and C genes the PCR results revealed negative. This technique also uncovered the presence of a surfactin and a mycosubtilin gene in *Bacillus pumilus* ATCC 14884. The origin of the isolates seems to be unrelated with the kind of lipopeptides detected. Hemolytic activity was observed in 29 isolates.

We assessed by a rapid, simple and efficient PCR methodology the presence of NRL genes in *B. pumilus*. This is the first report demonstrating the distribution and diversity of lipopeptides encoding genes in *B. pumilus* from different sources. The use of a rapid, simple and efficient PCR mehodology for detection of NRL it is of outmost relevance for assessing the strain potential for NRL production and toxigenicity.

Keywords *Bacillus pumilus*; non-ribosomal lipopeptides; PCR

Evidences of antibiotic activity of *Bacillus amyloliquefaciens* strain HNA3 isolated in a surgery room: preliminary findings

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A *Bacillus amyloliquefaciens HNA3* strain producing antibiotic compounds against both Gram positive and Gram negative bacteria had been isolated on a surgery room surface. The isolate produced a zone of inhibition against a *Bacillus subtilis* strain (taxonomically very close to *B.amyloliquefaciens*) growing on the same RODAC contact plate containing Plate Count Agar (PCA), provided by Oxoid ltd (as all the media used in this survey). Both strains had been identified genetically (NCBI nucleotide Genbank). Firstly, we tested *B.subtilis* in-vitro susceptibility to 13 antibiotics (chosen among the most commonly used in medical practices) and to 5 disinfectants. The results showed antibiotic resistance to cefotaxime and aztreonam while sodium hypocloride 5% had been the most effective disinfectant. *Bacillus amyloliquefaciens HNA3* isolate had been firstly suspended in 6 ml of Buffered Peptone Water (BPW) (pH=7,0), incubated at 37°C for 6-8 hours and then plated using a 5µl loop on Tryptic Soy Agar (TSA). This procedure had been repeated several times to obtain some pure cultures. Then, we tested in-vitro ability of *B. Amyloliquefaciens HNA3* to inhibit the growth of several bacterial species belonging both to Gram positive and Gram negative groups. 100 µ l of a suspension 0,5 Mac Farland in Tryptic Soy Broth (TSB) was seeded on the dried surface of a plate containing Mueller Hinton Agar (MHA). Then, an amount of a *B. amyloliquefaciens HN3* colony was taken from a pure culture with a 1 µl loop and placed at the centre of each plate previously seeded with a test microorganism. After 24 hours of incubation at 37°C, plates were examined. ATTC strains had been used for in-vitro susceptibility tests, even though some environmental isolates had been included too. In fact, unlike *B.subtilis* isolate, *B.subtilis* ATTC 6633 growth wasn't affected by *B.amyloliquefaciens HN3*. So, we repeated the same procedure on a Gram negative bacteria, *Aeromonas hydrophyla*, testing the susceptibility of both ATTC strain (ATCC 7966) and an environmental isolate obtaining a distinct zone of inhibition respectively of 15 mm and 14 mm in diameter. *Pseudomonas aeruginosa* ATCC 15442 and a *Vibrio harvey* environmental strains had been susceptible to *B. amyloliquefaciens HN3*. No zone of inhibition had been detected in *Staphylococcus aureus* (ATTC 6538 and ATTC 25923), *Streptococcus faecalis* (ATCC 33186), *Escherichia coli* (ATTC 10536), *Salmonella tiphymurium* (ATCC 14028), *Shigella sonnei* (ATCC 25931), *Klebsiella pneumoniae* (ATCC 13883) cultures. The results of further tests carried out on six different *Vibrio* environmental strains (including *Vibrio cholerae* and *Vibrio vulnificus*) showed no evident effects on the microbial growth. Nevertheless, the susceptibility of a *Vibrio* ATTC strain and of further *Pseudomonas* and *Aeromonas* strains will be also investigated.

The effect of supernatant liquid of a centrifuged *B.amyloliquefaciens HN3* 0,5 Mac Farland suspension in TSB had been also evaluated with negative results on all the strains previously tested. More deep studies are needed in order to isolate, to characterize the antimicrobial compounds and to gain informations about their synthesis. Previous researches had been demonstrated the ability of *B. amyloliquefaciens* to produce lantibiotics against Gram positive bacteria (*Staphylococcus aureus* for example) and against fungi but, till now, there is no evidence of an antimicrobial activity against Gram negative bacteria. The production of antibiotics play an important role in the competition among different microbial strains for food and space (quorum sensing). It is possible, then, that *B.amyloliquefaciens HN3* in a hospital environment (like a surgery room) had to compete with a opportunistic pathogens like *Pseudomonas aeruginosa* (responsible of a great part of nosocomial infections) and *Aeromonas hydrophyla*, an emerging pathogen. The preliminary findings of our research led us to consider the environmental hospital as the candidate place for searching new antibiotic producers.

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Fungal evaluation on *Camellia sinensis* teas irradiated with different water activities

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Teas from *Camellia sinensis* L. Kuntze infusions are the most worldwide beverages consumed after water. The tea consumption benefits have been attributed to high concentrations of antioxidants and ability to scavenge free radicals, such as flavonoids, and usually are consumed as green, black, oolong and white tea. With the increasing of consumption, the manufacturing and commercialization of plants became a health public concern. Diverse factors such as pollution in irrigation water, air and soil, harvest conditions, handling, drying and storage are important to consider on natural products processing, because they can allow high levels of microbial contamination, sometimes pathogenic. The presence of potentially toxigenic fungi were found in several studies on these products, indicating a great potential for the presence of mycotoxins that can cause serious damage in different organs and body systems, leading sometimes to death. Ionizing radiation is one of the most effective means to disinfect dry food ingredients. This treatment can inhibit cellular life division, like microorganisms, and promote a molecular structural modification. Radiation can cause a variety of biochemical and physical effects on microorganisms. Once absorbed by a biological material, ionizing radiation may have direct or indirect action. The most damage in irradiated cell occurs by indirect action of radiation. This is due to the fact that most living cells presents a proximally 80% of water in its composition and the indirect effect is caused by the interaction of radiation with a molecule of water (radiolysis), generating several kinds of free radicals. The aim of this study is evaluate the fungal contamination in *Camellia sinensis* tea irradiated with different radiation doses and water activities. Samples will be irradiated in 60Co irradiator (Gammacell 220, Nordion Ltd., Canada) at doses of 0, 2.5, 5.0, 7.5 and 10.0kGy with three different water activities.

Keywords oolong tea; irradiation; *Camellia sinensis*; fungi

Goat and cow milks are natural reservoirs of antifungal lactic acid bacteria

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Fermented dairy products susceptible to fungal spoilage are usually preserved by chemicals that prevent their alteration and the growth of potentially toxigenic fungi. In recent years, the new European legislation demanding for less chemical preservatives together with consumer's expectation for minimally processed foods with minimal amounts of chemical preservatives has increased the interest for alternative strategies of food preservation. Replacing chemical preservatives by antifungal lactic acid bacteria (ALAB), used as bio-protective cultures on dairy products, appears to be a promising way to fulfil consumers and legislation demands. Facing the need for protective cultures adapted to dairy products, we wondered whether milk could be a natural reservoir of ALAB. The aim of this study was then to evaluate the biodiversity of ALAB in 27 raw milk samples from cow, goat and ewe collected during three successive weeks at three lactation periods (beginning, middle and end).

For each sample, total bacteria were enumerated on non selective medium, whereas lactic acid bacteria (LAB) were enumerated on 8 semi-selective media. After counting, around 12,000 colonies were overlaid with one of the four targeted fungi, *Penicillium expansum*, *Mucor plumbeus*, *Kluyveromyces lactis* or *Pichia anomala* on 8 semi selective media, for antifungal activity screening. Colonies active against *Mucor plumbeus*, *Kluyveromyces lactis* and *Pichia anomala* were isolated and their 16S rRNA gene sequenced for identification.

Milk samples, whatever their origin, were dominated by LAB cocci (presumptive lactococci and leuconostoc). Enterococci, *Lactobacillus* spp. and psychrotolerant LAB were present in lower concentrations ($P < 0.05$). In most media, cow milk samples harbored significantly lower plate counts ($P < 0.05$) than goat milk, whereas ewe milk counts lied in between. According to activity tests, the number of recovered antifungal colonies depended on milk origin, lactation periods, growth medium and targeted fungi. Among the 1,235 isolated antifungal colonies, 8% were recovered from ewe milk, whereas 43 % and 49 % were recovered from goat and cow milks, respectively. Most of them (1,057 isolates) came from MRS-based media and half of them (634) were recovered in cow and goat milks from end lactation. An increase in the percentage of active colonies grown on LAMVAB and MRS was noticed in parallel, for cow and goat milks. *P. expansum* was the most frequently inhibited fungi, followed by *M. plumbeus, K. lactis* and *P. anomala* in decreasing order. Isolates active against *P. expansum* were over-represented in ewe milk, the ones active against *M. plumbeus* and *P. anomala* were over-represented in cow milk in end and middle lactation periods respectively, whereas those active against *K. lactis* were over-represented in goat milk.

Among the 733 bacterial isolates that were sequenced, 0.4% belonged to *Lactococcus*, 1.9% to *Enterococcus*, 3.7 % to *Leuconostoc*, and 94% to the *Lactobacillus* genus. Lactobacilli were ranked into eight phylogenetic groups: *Lb. casei* (55%), *Lb. fermentum* (19%), *Lb. plantarum* (17%) and *Lb. buchneri* (7%). The remaining 2 % lactobacilli belonged to the *Lb. delbrueckii*, *perolens*, *salivarius* and *coryniformis* groups. The *Lb. casei* group, mainly isolated on LAMVAB medium, contained isolates active against all fungi but they were over-represented in the population targeting *M. plumbeus*. Isolates from the *Lb. plantarum* group, frequently isolated in MRS incubated at 10°C were over-represented in the population targeting *K. lactis*, and the ones belonging to the *Lb. buchneri* group, frequently isolated in MRS at pH 5.5, were over-represented in the population targeting *P. anomala*.

In comparison to ewe milk, milk samples from cow and goat harboured a high diversity of ALAB varying according to lactation periods. In the tested conditions, the great majority of active colonies belonged to the *Lactobacillus* genus. Among this antifungal bacterial population, it appeared that targeted fungal species differed according to the *Lactobacillus* group tested whose presence largely depended on lactation period and milk origin.

Keywords milk; antifungal lactic acid bacteria

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Gram-positive bacteriocins : a new structure-based classification

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Bacteriocins are ribosomally-synthesized peptides or proteins produced by a wide range of bacteria. The antimicrobial activity of this group of natural substances against foodborne pathogenic and spoilage bacteria has raised considerable interest for their application in food preservation. Classifying these bacteriocins in well-defined classes according to their biochemical properties is a major step towards characterizing these anti-infective peptides and understanding their mode of action. Actually, the chosen criteria for bacteriocins' classification lack consistency and coherence. So, various classification schemes of bacteriocins resulted various levels of contradiction and sorting inefficiencies leading to bacteriocins belonging to more than one class at the same time and to a general lack of classification of many bacteriocins. Establishing a coherent and adequate classification scheme for these bacteriocins is sought after by several researchers in the field. It is not straightforward to formulate an efficient classification scheme that encompasses all of the existing bacteriocins. In the light of the structural data, here we revisit the previously proposed contradictory classification and we define new structure-based sequence fingerprints that support a subdivision of the bacteriocins into 12 groups. This work lays down a resourceful and consistent classification approach that resulted in classifying more than 70% of bacteriocins known to date and with potential to identify distinct classes for the remaining unclassified bacteriocins. Identified groups are characterized by the presence of highly conserved short amino acid motifs. Furthermore, unclassified bacteriocins are expected to form an identified group when there will be sufficient sequences.

Keywords bacteriocins; gram-positive; classification; sequence analysis; phylogeny

HPLC analysis of cephalosporin C biosynthesized by mutated and non mutated strains of *Aspergillus* and *Acremonium* species

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Antibiotics are secondary metabolites synthesized by microbes, extremely important for the health of mankind. Cephalosporins are broad spectrum antibiotics, similar in structure to penicillin but more resistant to β-lactamases. *Aspergillus* and *Acremonium* species were screened for cephalosporin C biosynthesis. The effect of different media constituents (inorganic nitrogen sources, Sucrose concentration and concentration of DL-methionine) were thoroughly investigated on the screened fungi and it was found that ammonium sulphate 7.5 mg/ml, Sucrose 30 mg/ml and DL methionine 3mg/ml were most suitable for higher yields of CPC. The selected fungi were subjected to chemical mutation (400 µg/ml, ethyl methane sulphonate for 1 hour for strain improvement hence enhanced biosynthesis of cephalosporin C. the spectrophotometric and HPLC analysis of the fermented broth were done for the quantification of the cephalosporin C yielded, using ceftriaxone as (2.5 mg/ml) as stsndard. *Acremonium kiliense* FCBP # 162 and *Acremonium furcatum* FCBP # 409 gave 2.583 mg/ml and 2.346 mg/ml cephalosporin C respectively. The bioassay analysis (antibacterial activity) was done using *E.coli* as test organism.

Key words: Cephalosporins; HPLC; β-lactamases

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Identification of novel antibiotic complex with broad-spectrum activity produced by *Lactococcus lactis* subsp. *lactis* strain 194

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Lactococcus lactis belongs to diverse group of lactic acid bacteria which have been used by mankind for thousands of years. They have been used as starter culture for preparation of fermented dairy products. It is well known, that *Lactococcus lactis* have antagonistic properties against some groups of microorganisms. The property serves to extend the shelf-life of the product. In general antagonistic activity of lactococci is provided by synthesis of lactic acid, diacetyl and bacteriocins. Bacteriocins are protein substances that exhibit antibacterial activity against closely related species. Bacteriocins can inhibit very restricted spectrum of bacteria, mostly limited to gram-positive bacteria. Different bacteriocins differ from each other by chemical structure and spectrum of antagonistic activity. *Lactococcus lactis* produces such bacteriocins as lacticin 481, lacticin 3147, several forms of nisin and lactococcins. Nisin is the most studied bacteriocin which is allowed for application as food preservative by European Parliament and Council (code E234). Recently it was revealed that some lactococci strains have an ability to produce antifungal substances which were determined as peptide or low-molecular phosphoglycolipid. For this reason lactococci can be considered as potential producers of different antimicrobials with wide activity spectrum.

Early a new strain *L. lactis* subsp. *lactis* 194 was isolated from fresh milk of Buryatia Republic (Russia). The strain demonstrated inhibitory activity against gram-positive, gram-negative bacteria and fungi *Aspergillus* genera and in a less degree against *Candida*. The strain was revealed to produce antibiotic complex, consisting of five components (A, B, C, D, E). Methods for isolation and purification of antibiotic substances have been developed to obtain individual components of the complex as chromatographically pure preparations. In order to separate the antibiotic substances into fractions and obtain chromatographically pure components, the aqueous concentrate was extracted with water-immissible organic solvents, after which the extracts were pooled, and the solvent was evaporated on a rotary evaporator to yield a dry residue.

The investigations were carried out to determine physicochemical properties of the components of the antibiotic complex. The individual antibiotic substances differ from each other by molecular mass, R_f values and biological properties. The major activity was defined by antibiotic substances specified as A, B, C, E. Component A was a hydrophobic molecule with molecular mass 879 Da that inhibited growth of gram-positive and gram-negative bacteria. It was shown to be an aromatic substance with alkyl groups. Component B was a hydrophobic substance which was responsible for antifungal activity of the strain. The molecule contained aromatic groups, keto-, aldehydic and alkyl residues. Antimicrobial substances A and B, in concordance with UV-spectrum, molecular mass and spectrum of antimicrobial activity were identified as novel antibiotics, that were absent in Berdy database BNPD.

Component D was a positively charged substance identified as peptide built of the following amino acids: asparagine, glutamine, serine, glycin, alanine, lysine, ornithin in ratio 1:1:1:1:1:2:3. Component D inhibited growth of gram-positive bacteria only and differed from nisin by molecular mass and electrophoresis migration.

Keywords: *Lactococcus lactis* subsp. *lactis* 194, antibiotic complex, novel antimicrobial substances.

In vitro antagonist activity of Mexican *Trichoderma* spp. isolates against *Phytophthora capsici* causing pepper wilt.

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P. capsici is a plant pathogen with a wide range of solanaceae and cucurbitaceae hosts. In Mexico, this disease can be devastating in the pepper resulting losses from 60 to 100%. Traditionally it has been used fungicides to control *P. capsici*; however, with the frequent use of these pesticides, some *Phytophthora* species have developed resistance to them. A natural alternative for controlling this plant pathogen that does not affect human health and have no effect on environment is the use of antagonists such as *Trichoderma spp.* This study was carried out to determined the in vitro antagonistic effect of 31 strains of *Trichoderma* isolated from different Mexican regions on *P. capsici*. The effect of volatile compounds and the activity of toxic substances produced by *Trichoderma* on *P. capsici* were evaluated using the scale reported by Bell et al. (1982). In addition were determinate the days to contact among *Trichoderma* strains and *P. capsici* and the percentage of inhibition of plant pathogenic development. The data in percentage were transformed by arc sin $\sqrt{x}$ +1.The data were subjected to analysis of variance and differences between treatments were analyzed by the Tukey test in the SAS statistical program. It was observed that 13 strains of *Trichoderma* showed the most antagonistic effect (level 1of the Bell scale), where the antagonist overgrows the mycelium of *P. capsici* and covers the entire surface of the culture medium. The period of contact between *Trichoderma* and *P. capsici* was two days for 30 of the 31 strains. The P. capsici mycelial growth inhibition by the *Trichoderma* volatile compounds ranged from 4.3 to 48.8%. All *Trichoderma* strains were able to produce volatile compounds, the highest *P. capsici* mycelial growth inhibition effect was observed with *T. asperellum* strains. The inhibitory effect on *P. capsici* of toxic substances produced by 14 strains of *Trichoderma* ranged from 0 to 15.29%. It was observed that not all *Trichoderma* strains presented antagonist activity on *P. capsici.* Seven *Trichoderma* straing with the highest production of toxic compounds were identify as *Trichoderma asperellum*, one as *T. hamatum.* One strain of *T. rossicum* study showed no effect on *P. capsici*. In general, considering the evidence of antagonism studied, 13 *Trichoderma* strains have good level of antagonism on *P. capsici.*

Key words: mycelia inhibition, volatile compounds, toxic substances.

In vitro studies of *Trichoderma* spp. as biological control agents for potato black scab (*Rhizoctonia solani*).

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R. solani which causing of the black scab of potato is an important plant pathogen in Mexico, its incidence may causes lost up to 35% in stems and 97.7% in tubers. The battle with fungicides such as pencycuron has promoted that some *R. solani* stain develop resistance to this fungicide. The biological control using microorganisms such as *Trichoderma* is a viable option for *R. solani* management. Therefore, we evaluated *in vitro* the antifungistatic effect of 31 *Trichoderma* isolates on three strains of *R. solani* pencycuron resistant. The effect of *Trichoderma* volatile compounds on *R. solani* were evaluated using the scale proposed by Bell et al. (1982), In addition were evaluated the days to contact among *Trichoderma* strains and *R. solani* and the percentage of *R. solani* mycelia inhibition as effect of *Trichoderma* strains volatile compounds. The data in percentage were transformed with arcsin $\sqrt{x}$ +1. The data were subjected to analysis of variance and differences between treatments were analyzed by the Tukey test in the SAS statistical program. The results showed that the number of days to the first contact between the antagonist and the plant pathogenic fungus varied from one or two depending on the *Trichoderma* strain. It also was noted that 14 of 21 strains of *Trichoderma* are located in the level 1 of the Bell et al., scale (1982) (*Trichoderma* overgrows *R. solani* and covers the entire surface of the medium). Two strains of *R. solani* were placed in level four of the Bell et al scale (plant pathogen growth overgrows *Trichoderma*), filling ¾ of the Petri dish. The evidence on *Trichoderma* volatile compounds production indicated that four *Trichoderma* strains inhibited the mycelia growth of *R. solani* by 50%. The above results indicated that the use of *Trichoderma* is viable ecological option for management of *R. solani* resistant to fungicides in potato.

Key Word: Inhibition mycelia, antagonism, *Trichoderma spp, Rhizoctonia solani*

Inhibition of fungal phytopathogens by lipopeptide biosurfactants produced by Gram-positive bacterial endophytes.

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Microbial endophytes reside in the living tissues of plants and may proctect their host by producing a lot of natural products (Strobel et al. 2004; Cho et al., 2007). Microbial compounds exhibiting antagonistic activities include a wide variety of surface active compounds suche as lipopeptides, glycolipids and phospholipids. Several studies show the activity of lipopeptides such as surfactin, iturin and fengycin against fungal phytopathogens (Tourè et al. 2004, Ongena et al., 2005, Gong et al., 2006; Tendulkar et al, 2007). The aims of this work was to isolate Gram-positive bacterial endophytes, from oleander (*Nerium oleander*), rice (*Oryza sativa*), and acacia (*Robinia pseudoacacia*) stems, able to produce surface active compounds with inhibitory activity against a variety of phyto-pathogenic fungi. Bacterial endophytes were isolated from the inside of cutted sterilized stems on 1/10 stenght Trypticase Soy Agar added with cycloheximide. A total of 30 Gram positive spore forming bacterial colonies were isolated. The oil spreading assay on the supernatants showed that 11 isolates were able to produce biosurfactants, four were from *R. pseudoacacia* (AB1, AC5, AC7, AG1), 5 from *N. oleander* (OA1, OA3, OC4, OC5), and 2 from *O. sativa* (RA1,RE1). Extracted biosurfactants reduced superficial tension of water approximately between 28.6 and 36 mN/m and their CMC varied between 14.7 and 129.5 µg/ml. IF-TR analysis of the extracted biosurfactants showed that they belongs to the lipopeptides family. Antifungal activity of purified biosurfactants was tested against the following phytopathogenic fungi: *Botrytis cinerea, Cercospora beticola, Helminthosporium teres, Microdochium nivale, Phytophtora infestans, Pyricularia oryzae, and Rhizoctonia solani*. All the fungi, with the exception of *Phytophtora infestans*,were inhibited. At the concentration of 1250 µg/ml, percentages of inhibition of fungal growth were between 50 to 100%. Glasshouse experiments of plant protection against *Botrytis cinerea* with biosurfactant AC7 are on going.

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Key words: endophytes; lipopeptide biosurfactants; growth inhibition; phytopathogens.

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Insights into the *in situ* antilisterial properties exerted by a complex cheese ecosystem

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The foodborne pathogen *Listeria monocytogenes* poses a high risk for smear cheeses producers. The development along ripening of the microbial mat typical of smear cheeses, composed of de-acidifying yeasts and lactate-metabolizing bacteria, creates favorable conditions for *Listeria* growth in terms of pH and nutrients. Growth of *Listeria* is thereby intimately linked to the composition of the smear and to its development. Natural complex smear ecosystems exhibiting antilisterial activity have already been described, but the mechanisms involved still need to be elucidated. Smear ecosystems may contain as much as 15 different species, showing either promoting or inhibiting effects on *Listeria* growth, and these complex interactions will determine the fate of the pathogen.

Population dynamics of a Raclette smear ecosystem exhibiting strong *in situ* antilisterial activity was investigated. This study enabled the detection, isolation and testing of *in situ* inhibition properties for three Facultative Anaerobic Halophilic and Alkaliphilic (FAHA) species, i.e. *Marinilactibacillus psychrotolerans* ALK 9, *Alkalibacterium kapii* ALK 6, and *Facklamia tabacinasalis* ALK 1, the two latter species being described for the first time in a smear ecosystem. Early development of FAHA species observed in the complex smear, i.e. at day 15, could be reproduced on test cheeses inoculated with FAHA species and 6 control species also isolated from the complex smear. Growth of the FAHA species, inoculated either as single or mixed cultures, was correlated with a 1 to 2-log decrease in *Listeria* cell counts, compared to cheeses inoculated with the control species only. The inhibition was not as powerful as in the original smear. Development of the control species was however not identical to the dynamics observed in the complex ecosystem, and this altered smear development may have exerted a promoting effect on *Listeria*.

These results suggest that early development of FAHA species is contributing to the inhibition properties of the smear ecosystem investigated. This work highlights the potential of population dynamics studies to elucidate the microorganisms involved in pathogen inhibition by complex ecosystems and to develop new protective systems of biopreservation based on this new acquired knowledge.

Keywords *Listeria*; smear ecosystem; *in situ* antimicrobial activity; complex interactions; population dynamics

Inter-species interactions modifies antibiotic production pattern in *Streptomyces coelicolor*

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In this study we report how *Streptomyces coelicolor* altered its antibiotics production strategy when challenged with other bacteria. The antibiotics studied were red-pigmented undecylprodigiosin and blue-pigmented actinorhodin. In the defined medium, pure cultures of *S. coelicolor* produced mainly actinorhodin and only low quantities of undecylprodigiosin. Upon interaction with *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus*, *S. coelicolor* changed its antibiotics production pattern and increased undecylprodigiosin production whereas actinorhodin was suppressed. Furthermore, an increase in the production of undecylprodigiosin was observed at an earlier point in the exponential phase compared with the pure culture. In the preliminary test of the antimicrobial activities of the extracted antibiotics, undecylprodigiosin always inhibited the growth of *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus* whereas actinorhodin was less effective. Our work contributes to the new approach of exploiting microbial interactions as strategies for screening programmes to discover novel bioproducts and to increase the production of known bioactive compounds.

Key words: *Streptomyces coelicolor*, Inter-species interactions, undecylprodigiosin, actinorhodin

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Isolation and Screening of Novel Antibiotic Producing *Streptomyces* from Southwest Turkey Soils

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The purpose of this study is to explore secondary metabolites belonging to *Streptomyces* which show antibiotical activities on many gram negative and gram positive bacterial pathogens including *Stenotrophomonas maltophilia* and *Staphylococcus* which are multi-resistant to antibiotics. For this aim, a total of 234 different *Streptomyces* isolates were recovered from soil samples taken from areas with various vegetation types in Mugla and its surrounding for their antimicrobial potential. All the isolates were screened with spektra-plak method regarding the production of antibacterial metabolites. The bacteria were identified on the basis of morphological characterization (presence or absence of sporangia in aerial mycelium and/or in the substrate mycelium, fragmentation of substrate mycelium, absence of either aerial or substrate mycelium). Aerial mycelium, substrate mycelium and diffuse pigment colours of all isolates were grouped by NBS/ISCC colour system.

In this screening process, the target bacteria chosen from pathogens which are ten clinically-significant gram positive and nine gram negative bacteria. Out of these, 61 isolates exhibited inhibitory activity against at least one of the tested microorganisms. Approximately, 74% isolates produced antibacterial substances against Gram positive bacteria, 47% against Gram negative bacteria. The results indicated that 26 isolates were highly active against at least one clinically significant multi-resistant *Staphylococcus spp.* with an inhibition zone at ≥20 mm.

Keywords: *Streptomyces*, antibiotic producing, *Staphylococcus, Stenotrophomonas*

Management of corm-rot disease pathogen of gladiolus by plant extracts

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A pot experiment was conducted to evaluate the efficacy of aqueous extracts of six plant species viz. *Azadirachta indica* A. Juss. (neem), *Alstonia scholaris* (L.) R. Br., *Lawsonia alba* Lam., *Allium cepa* L., *Allium sativum* L. and *Zingiber officinale* Roscoe, and a systemic fungicide carbendazim 50% (w/w)WP to manage the corm rot disease of gladiolus [*Gladiolus grandiflorus* L.] caused by a fungal pathogen *Fusarium oxysporum* f.sp. *gladioli* (Massey) Snyd. & Hans. *Fusarium* inoculation showed 80% disease incidence with 54 disease lesions per corm. Recommended dose of the chemical fungicide carbendazim significantly reduced the disease incidence to 13% and number of lesions to 6 per corm. Plant extract treatments exhibited variable effects on incidence and severity of disease. In general, all the test plant extracts managed the corm rot disease to some extent. Aqueous bulb extracts of *A. sativum* and *A. cepa* and rhizome extract of *Z. officinale* showed better disease management potential than that of recommended dose of carbendazim. *Fusarium* inoculation significantly declined shoot growth. In general, carbendazim as well as aqueous extracts enhanced shoot growth to variable extents as compared to *Fusarium* control.

Management of *Sclerotium rolfsii* by *Coronopus didymus*

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Present study was carried out to investigate the antifungal activity of different parts of *Coronopus didymus* for the management of Sclerotium wilt of bell pepper (*Capsicum annuum* L.) caused by fungus *Sclerotium rolfsii*. In laboratory bioassays, the effect of methanolic extracts of 0.5, 1.0 and 1.5% of different parts of *C. didymus* was investigated against growth of *S. rolfsii*. Leaf, root and inflorescence extracts of 1.0% concentration and above significantly reduced the fungal biomass by 29–67%. Methanolic inflorescence and leaf extracts were combined and successively extracted with n-hexane, chloroform, ethyl acetate and n-butanol in increasing order of polarity. Antifungal bioassays revealed that ethyl acetate and butanol fraction were highly effective in suppressing the growth of the fungus. In pot trials, soil was amended with dried shoot powder of *C. didymus* at 0.5, 1.0 and 1.5 g 100 g^{-1} of soil. All the soil amendment treatments markedly reduced disease incidence and mortality percentage compared to control. Effectiveness of the soil amendment was parallel increased by increasing the quantity of plant material.

Mode of action of bacteriocin ST8SH on *Listeria ivanovii* subsp. *ivanovii* ATCC 19119 and *Enterococcus faecalis* ATCC 19443

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Bacteriocins are ribosomally synthesized antibacterial peptides and are usually active against genetically related species. They have been grouped into 4 classes based on their structure and mode of action. In the last two decades several reports were focused on the production of bacteriocins from lactic acid bacteria isolated from different ecological niches including fermented products, vegetables, fruits, meat, fish, human and animal GIT. Their bactericidal mechanisms may include pore formation, degradation of cellular DNA, disruption through specific cleavage of 16S rDNA, and inhibition of peptidoglycan synthesis. Many LAB produce bacteriocins with rather broad spectra of inhibition. Several LAB bacteriocins have been studied and they offer potential applications in food preservation.

Strain ST8SH, isolated from Bulgarian salami was identified as *Lactobacillus plantarum* based on biochemical tests, sugar fermentation reactions (API50CHL), PCR with species-specific primers and 16S rDNA sequencing. Strain ST8SH produces a c.a. 3.0 kDa (based on tricine-SDS-PAGE) pediocin-like bacteriocin (based on PCR analysis and presence of pediocin gene), active against *Streptococcus caprinus*, *Streptococcus* spp. *Listeria innocua*, *Listeria ivanovii* subsp. *ivanovii*, *Lactobacillus casei*, *Lactobacillus curvatus*, *Lactobacillus salivarius, Lactobacillus pentosus*, *Enterococcus mundtii*, *Enterococcus faecalis* and *Lactococcus lactis* subsp. *lactis*. Peptide ST8SH adsorbs at low levels (400 AU/ml) to producer cells. High cell numbers of *Lactobacillus plantarum* ST8SH and *Listeria ivanovii subsp. ivanovii* ATCC 19119 were recorded at beginning when co-cultured. However, the cell numbers of *Listeria ivanovii subsp. ivanovii* ATCC 19119 decreased from $2.71x10^4$ CFU/ml to $1,62x10^2$ CFU/ml in 12h and to undetectable levels after 24h. Bacteriocin ST8SH production was stimulated by presence of *Listeria ivanovii* subsp. *ivanovii* ATCC 19119. Similar results were obtained with addition of 10% autoclaved overnight culture of *Listeria ivanovii subsp. ivanovii* ATCC 19119 to MRS growth media on production of bacteriocin ST8SH. Addition of bacteriocin ST8SH to the exponential or stationary phase culture of *L. ivanovii* subsp. *ivanovii* ATCC19119 and *E. faecalis* ATCC 19443 inhibited growth for 24h. The effect of bacteriocin ST8SH on cells of *L. ivanovii* subsp. *ivanovii* ATCC19119 was visualised by AFM and indirectly recorded based on enzyme, protein and nucleotide material leakage.

Keywords: *Lactobacillus plantarum*, salami, bacteriocin

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Molecular identification of a bacteriocin-like inhibitory substance produced by *Enterococcus faecium* MXVK29.

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Enterococcus faecium MXVK29 is a lactic acid bacterium (LAB), isolated from Mexican fermented sausages, that produces a class IIa bacteriocin, according to its N-terminal amino acid sequence. Class IIa bacteriocins produced by LAB are an interesting group of antimicrobial peptides for use in food preservation, because they can inhibit spoilage and pathogenic bacteria, such as *Listeria spp., Enterococcus faecalis, Lactobacillus spp.*, and *Staphylococcus spp*. This study aimed to identify the structural gene for the production of a bacteriocin-like inhibitory substance (BLIS) in *Enterococcus faecium* MXKV29, and to compare it with others previously studied BLIS. Genomic DNA of *Enterococcus faecium* was extracted from an overnight culture by using the Wizard Genomic DNA Purification Kit (Promega). Purified total DNA was used as a DNA template for PCR amplification, by screening for six published structural genes for enterocins (*entA*, *entB*, *entP*, *entL50A/B*, *ent1071AB*, and *entQ*). PCR amplifications were carried out using an initial denaturation step (5 min at 95°C), followed by 30 cycles of denaturation (95°C for 1 min), annealing (56°C for 1 min), and elongation (72°C for 1 min), and ending with a final extension step (72°C for 10 min). The annealing temperature was determined using a temperature gradient (56°C ± 6°C) for each gene. After amplification, PCR products were examined on 3% agarose gel electrophoresis followed by ethidium bromide staining. The amplified product was purified using the Wizard SV Gel and PCR Clean-Up Kit (Promega). Purified fragments were sequenced, and sequences were compared using the NCBI database with the BLAST homology search (www.ncbi.nlm.nih.gov/BLAST/). Two PCR products were obtained from the primers that encoded enterocin A (*entA*) and enterocin B (*entB*). The amplified fragments had a size of around 150 and 200 pb, respectively, and they were purified and sequenced. The obtained nucleotide sequences showed 96% similarity to the genes encoding for published enterocin A and B, and the predicted amino acid sequence was 100% similar to the mature enterocin A (KYYGNGVYCTKNKCTVDWAKATTCIAGMSIGGFLGGAIPGKC) and enterocin B (HRMPNELNRPNNLSKGGAKCGAAIAGGLFGIPKGPLAWAAGLANVYSKCN). These results indicated that *Enterococcus faecium* MXVK29 produces both enterocins A and B, belonging to class II bacteriocins. This group of bacteriocins has a great potential to be used as antimicrobial substances in food, but more studies should be performed to develop applications for them.

Keywords: bacteriocin; BLIS; enterocin; Enterococci; food-preservative

Nisin-like bacteriocin produced by a *Lactococcus lactis* strain isolated from charqui, a Brazilian fermented, salted and dried meat product

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Charqui is a traditional fermented, salted and dried meat product from Brazil. Despite the low Aw (c.a. 0.70), halophilic microorganisms can grow in charqui and cause spoilage. The growth of these undesired bacteria can be inhibited by antimicrobial components produced by lactic acid bacteria isolated from the product, as they are part of the natural microflora, and play an important role in the sensorial characteristics of charqui. Many of these lactic acid bacteria produce antimicrobial peptides (bacteriocins) that cause pore formation in the microbial cytoplasmic membrane, degradation of cellular DNA, disruption through specific cleavage of 16S rDNA, and inhibition of peptidoglycan synthesis.

In this work, we report results on the isolation of a bacteriocinogenic strain from charqui (*Lactococcus lactis* 69) and characterization of the bacteriocin produced by this strain. The antimicrobial compounds produced by this strain were sensitive to the treatment with proteolytic enzymes, but were not affected by heating at 100ºC for 120 min, extreme pH (2 to 12) and chemical agents (SDS, EDTA, Tween 80 and urea), confirming that the activity was due to bacteriocin. In *vitro* tests showed that the bacteriocin was active against halophilic bacteria, isolated from spoiled charqui, in addition to other important food spoilage and pathogenic microorganisms (*Listeria monocytogenes*, *Staphylococcus aureus* and several lactic acid bacteria). Genetic studies indicated that the bacteriocin is identical to nisin Z, presenting differences in the leader peptide when compared to other nisin-like prepeptides. *L. lactis* 69 was able to grow and produce bacteriocin in culture media with high NaCl concentrations, similar to those found in charqui (20%). Regarding the mode of action, the treatment of stationary phase cells of *L. monocytogenes* ScottA (10^9 CFU/ml) with the bacteriocin resulted in complete cell inactivation. After 1h contact time, no viable cells of *L. monocytogenes* ScottA were detected, while no significant changes in cell numbers were recorded in the untreated (control) sample. Besides, addition of this bacteriocin to a 3-h-old and 7-h-old culture of *L. monocytogenes* ScottA caused cell death as no viable cells were detected after 25h , suggesting that the mode of action of this bacteriocin is bactericidal. Adsorption tests to *L. monocytogenes* Scott A were also performed, and carried out at 4, 25, 30 and 37ºC, at pH 2, 4, 6, 8 and 10, and in the presence of selected chemicals (NaCl, tween, glycerol and SDS). The highest adsorption to *Listeria monocytogenes* Scott A was recorded at 4ºC, pH 2 and 4 and in presence of NaCl. The results observed in this work indicate that the bacteriocinogenic strain *L. Lactis* 69, isolated from charqui, presents an interesting potential for application in the biopreservation of this product. Further studies with charqui added of this bacteriocinogenic strain are under development.

Keywords biopreservation; salted and fermented meat products; bacteriocins

Phage-derived antimicrobials against food-borne *Staphylococcus aureus*

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Staphylococcus aureus is an opportunistic bacterial pathogen that has a large impact on the food-processing sector. It is one of the main etiological agents of food poisoning due to biosynthesis of heat-stable enterotoxins in food. In dairy products, the presence of *S. aureus* is usually associated with post-pasteurization contamination owing to improper handling of the product, but also with contamination of raw milk from animals with subclinical mastitis as *S. aureus* is one of the main causes of mastitis in cattle.

Bacteriophages are a promising approach to the biocontrol of pathogens in food since they act as bactericidal agents by inducing the lysis of their host. In addition, several phage-encoded antimicrobial activities could also be applied as food biopreservatives.

We have isolated from the dairy environment two phages, phi-SauS-IPLA35 and phi-SauS-IPLA88, active against bovine *S. aureus*. The characterization and genomic analysis of these phages, belonging to the *Shiphoviridae* family, revealed their genome size (45,344 bp and 42,526 bp, respectively), their modular gene organization (containing 62 and 61 *orf*s, respectively) and DNA packaging mechanism. No relevant virulence traits were detected (García et al., 2009a). Challenge assays demonstrated that a mixture of the two phages was able to kill *S. aureus* in pasteurized milk and curd manufacture (García et al., 2007; 2009b). Moreover, combination of phages with nisin, a bacteriocin commonly used as a biopreservative in dairy products, enhanced their antimicrobial activity although nisin resistant strains may hinder phage infection (Martínez et al., 2008).

Bioinformatic analysis of the phage genomes revealed muralytic activities associated with structural components (peptidoglycan hydrolase) and others included in lysis cassettes (endolysin). We have characterized the antimicrobial activity of the endolysin protein (LysH5) from the bacteriophage phi-IPLA88. This protein was able to kill rapidly *S. aureus* growing in pasteurized milk (Obeso et al., 2008). A strong synergy between nisin and LysH5 was observed (García et al., 2010).

Currently, we have approached the characterization of the 72.5 kDa virion-associated peptidoglycan hydrolase, HydH5, from bacteriophage phi-IPLA88. Bioinformatic analysis of HydH5 aminoacidic sequence revealed that this protein has two catalytic domains: CHAP (15-149 aa) and LYZ (483-629 aa), both of them related to peptidoglycan hydrolysis. Both complete HydH5 protein and truncated HydH5-derived proteins containing only one of each catalytic domain were overexpressed in *E. coli* and purified. These proteins bind *S. aureus* Sa9 cells and lysed them as shown by zymograms and viability tests supporting the use of HydH5 as a novel food biopreservative. Challenge assays in milk are currently in progress.

Overall, our work shows that both phages and phage-derived products are useful tools for developping novel biocontrol strategies supplying new hurdles to enhance the hygienic quality of milk and dairy prodcuts.

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Keywords: Food biopreservation, phages, peptidoglycan hydrolysis, *Staphylococcus aureus*

Probiotic potential related to bacteriocinogenic activity of two lactic acid bacteria isolated from goat milk in the presence of prebiotic carbohydrates

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Potentially probiotic strains able to produce antimicrobial bacteriocins can contribute to the resistance against colonization of the gastrointestinal tract with pathogens and benefit the host's health. The objective of this work was to evaluate the probiotic potential of two bacteriocinogenic strains isolated from goat milk and identified as *Lactococcus lactis* subsp. *lactis* (strain DF4Mi) and *Lactobacillus paracasei* subsp. *paracasei* (strain DF60Mi) when grown in the presence of prebiotic carbohydrates (fructooligosaccharides –FOS, galactooligosaccharides - GOS and inulin). Microbiological and molecular methods were used to study the production of bacteriocins. The capability to survive simulated gastrointestinal conditions, the spectrum of activity of the bacteriocins and the influence of medicaments on the growth of the two strains were also evaluated. Both strains were grown in glucose-free MRS pH 6.5, supplemented with 20g/L of one of the testing carbohydrates, plus a control (MRS with glucose 20g/L), during 24h at 30ºC. After 0, 4, 8, 12 and 24h of incubation, samples were withdrawn to measure cell viability, pH and bacteriocin activity by the "spot-on-the-lawn" method. Other experiments tested the growth of the strains in MRS broth with different initial pH and in the presence of ox-bile, during 24h at 30ºC. Both strains grew well in media containing GOS, and the amount of produced bacteriocins (3200 AU/ml) was similar to that in media with glucose (control) ($P>0.05$). In contrast, FOS and inulin did not support bacterial growth or production of bacteriocin. Good growth of DF4Mi and DF60Mi was recorded in MRS broth supplemented with 0.6% and 0.2% (w/v) ox-bile and also in MRS broth adjusted to pH 5.0 – 11.0 and pH 6.0 – 9.0, respectively. The pH influenced the production of bacteriocins, as no activity was detected when both strains were cultivated at pH 3.0, 4.0 or 5.0. Bacteriocins produced by strains DF4Mi and DF60Mi were able to inhibit several *Listeria monocytogenes* and *Staphylococcus aureus* isolated from foods. Among a number of antibiotics tested, DF4Mi was resistant to trimethoprim and nalidixic acid and DF60Mi was resistant to vancomycin and tobramicin. The inhibitory effect of medicaments on growth of DF04Mi and DF60Mi was recorded for Amoxil (MIC < 0.2 mg/ml), Atlansil (MIC 1.25 mg/ml), Cataflam (MIC 2.5 and 1.25 mg/ml, respectively), Potassium Diclofenac (MIC 0.16 - 0.32 and 1.25 mg/ml, respectively), Spidufen (MIC 7.5 and 15.0 mg/ml, respectively) and Urobel (MIC 2.5 mg/ml). Preliminary molecular studies, using RAPD-PCR, on genomic DNA extracted from the strain DF60Mi (bacteriocin producer and non-bacteriocin producer) combined to both eletrophoretical plasmid profiles suggest that production of bacteriocins by strain DF60Mi may be encoded by plasmidial genes. In conclusion, both strains present probiotic potential based on their growth and bacteriocin production in the presence of prebiotic GOS and resistance, to some extent, to gastrointestinal conditions. Further molecular studies are required for the understanding of the mechanisms of bacteriocins expression in these conditions.

Keywords bacteriocin; *Lactococcus lactis* subsp. *lactis*; *Lactobacillus paracasei* subsp. *paracasei*; probiotic; prebiotic

Production and characterization of enterocins from Enterococcus faecium IJ-06 and IJ-21 isolated from indigenous dairy products

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The study was conducted to investigate *Enterococcus faecium* IJ-06 (isolated from local processed cheese) and *Enterococcus faecium* IJ-21 (isolated from yoghurt) for their bacteriocin production potential, antimicrobial activity and relevant virulence traits. Isolated strains were non haemolytic, susceptible to vancomycin and possess genes for enterocin A (*entA*) as well as enterocin P (*entP*). Assessment of *vanA*, *vanB* genes and virulence determinants *gelE, agg, cyl* were done through PCR. Immobilization of isolated strains in Ca-alginate beads resulted in increased bacteriocin production and stability as compared with planktonic cell fermentation. Partial purification of the enterocin was carried out by ammonium sulphate precipitation followed by chloroform-methanol extraction and column chromatography that displayed 50 to 70 fold purification and increase in specific activity. The enterocin was inhibitory towards *Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and other enterococci. Characterization of enterocin with specific treatment followed by residual activity assay showed its thermostability with a wide pH range of activity and sensitivity to proteases. The molecular weight of partially purified enterocins were approximately 4.5 kDa as determined by SDS-PAGE. It is promising to investigate the safe exploitation of these strains or their enterocins for the control of undesirable microflora, especially *Listeria monocytogenes*, *Bacillus subtilis* and *Bacillus cereus* of food products for microbial food safety.

Production of anti-Listerial metabolites, Phenyllactic acid and Indolelactic acid in *Geotrichum candidum* ATCC 204307 by Phenylalanine and Tryptophan metabolism respectively

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The concept of naturally produced antimicrobial agents in food products is gaining considerable popularity ever since the consumers have become wary of the chemical additives and heavy processing employed in the food industry. To our advantage, the discovery of antipathogenic metabolites like Phenyllactic acid (PLA) and indolelactic acid (ILA) produced naturally by cheese starter culture microorganisms like *Geotrichum candidum* ATCC 204307 seems quite promising. However, further studies are required before their use as potential protective culture in cheese could be successfully established. Keeping this in view, we investigated the metabolic pathways involved in the production of these two compounds by utilising microculturing and HPLC methods. Using synthetic media, the role of phenylalanine (Phe) and tryptophan (Trp) was established. Presence or absence of intermediate metabolites like Phenylpyruvate (PPA) and indole pyruvate (IPA) was also verified in order to map out the pathway in detail. The amount of metabolites produced was directly related to the concentration of amino acid provided in the medium and the culture time. PPA has been found to be present as an intermediate reaction product in Phe conversion to PLA but similar relation could not be ascertained for Trp conversion to ILA.

Keywords protective cultures, Phenylalanine metabolism, Tryptophan metabolism, anti listerial compounds

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Resistance of *Lactococcus lactis* to the lipid II-binding bacteriocin Lcn972

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Bacteriocins are ribosomally-synthesised antimicrobial peptides. Most of those produced by lactic acid bacteria act as membrane permeabilizers making pores in the cytoplasmic membrane of target bacteria. However, some were shown to inferere with cell wall biosynthesis. This is the case for the lantibiotic nisin, which uses the cell wall precursor lipid II as a docking molecule for pore formation. Thereby, nisin combines two modes of action in the same molecule for potent antimicrobial activity: cell wall biosynthesis inhibition and pore formation (Wiedemann *et al*., 2001).

Our group has identified the lipid II-binding bacteriocin lactococcin 972 (Lcn972). Lcn972 is a non-modified 66-aa bacteriocin produced by *Lactococcus lactis* IPLA972. It has a narrow activity spectrum, inhibiting *Lactococcus* only. Lcn972 partially inhibits the incorporation of N-acetyl-glucosamine into cell wall peptidoglycan and blocks septum biosynthesis. Lcn972 specifically binds to lipid II and inhibits enzymes whose substrate is lipid II (Martínez *et al*., 2000; 2008).

In this work we have investigated molecular mechanisms underlying resistance to Lcn972. Strains resistant to this bacteriocin were isolated by growing *L. lactis* MG1614 at increasing Lcn972 concentrations. The MIC of Lcn972 for *L. lactis* MG1614 was 10 AU/ml while the MICs for the Lcn972^R strains *L. lactis* D1 and *L. lactis* D1-20 were more than 320 and 80 AU/ml, respectively. High resistance levels (MIC >320 AU/ml) were lost in the absence of selective pressure after 60 generations whereas the resistant phenotype of *L. lactis* D1-20 was stable over 100 generations.

Remarkable changes were observed in the muropeptide composition of peptidoglycan isolated from the Lcn972^R strains. Lcn972 resistance is associated with a higher content of muropeptides with tripeptide side chains whereas the content of those with pentapeptide chains decreased, suggesting a higher D-Ala-carboxypeptidase activity in the resistant strain. Despite of this, the crosslinking index was similar to that of the wildtype *L. lactis* MG1614. No differences in Triton X-100-induced autolysis rate were observed although Lcn972^R strains were more sensitive to the nonionic detergent and growth was inhibited in the presence of 0.006% Triton X-100.

Genome-wide transcriptomics revealed differences in gene expression between the Lcn972^R *L. lactis* D1 and its parent *L. lactis* MG1614. Fourteen genes were significantly up-regulated and 29 were down-regulated (expression change > 2-fold, $p<0.001$). Up-regulated genes included members of the cell envelope stress (CesR) regulon, the penicillin-binding protein *pbpX* and *llmg2447*, which may encode a putative ECF anti-sigma factor. Resistance to Lcn972 seemed to be also linked to down-regulation of genes involved in maltose metabolism. Absence of growth of the Lcn972^R strains in a chemically defined medium with maltose as the sole carbon source was experimentally confirmed. Lcn972 susceptibility tests with knock-out and overexpressing mutants of selected genes are currently in progress to establish their contribution to the Lcn972 resistance phenotype.

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Keywords bacteriocins; cell wall; *Lactococcus lactis*; resistance phenotype.

Strain improvement of Aspergillus and acremonium species for enhanced biosynthesis of cephalosporin

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Antibiotics are secondary metabolites synthesized by microbes, extremely important for the health of mankind. Cephalosporins are broad spectrum antibiotics, similar in structure to penicillin but more r4esistant to β-lactamases. *Aspergillus* and *Acremonium* species were screened for cephalosporin C biosynthesis. The effect of different media constituents (inorganic nitrogen sources, Sucrose concentration and concentration od DL-methionine) were thoroughly investigated on the screened fungi and it was found that ammonium sulphate 7.5 mg/ml, Sucrose 30 mg/ml and DL methionine 3mg/ml were most suitable for higher yields of CPC. The selected fungi were subjected to chemical mutation (400 µg/ml, ethyl methane sulphonate for 1 hour for strain improvement hence enhanced biosynthesis of cephalosporin C. the spectrophotometric and HPLC analysis of the fermented broth were done for the quantification of the cephalosporin C yielded, using ceftriaxone as (2.5 mg/ml) as stsndard. *Acremonium kiliense* FCBP # 162 and *Acremonium furcatum* FCBP # 409, gave 2.583 mg/ml and 2.346 mg/ml cephalosporin C respectively. The bioassay analysis (antibacterial activity) were done using *E.coli* as test organism.

Structural Model for a Holin-like Protein Tmp3 Exhibiting Broad Spectrum Antibacterial Activity

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In recent years, phage encoded lytic proteins (virolysins) have gained much attention as an alternate to antibiotics in combating antibacterial resistance among pathogens. Well studied virolysins include various classes of endolysins encoded by bacteriophage genomes. Holins are essential lytic proteins encoded by phage genome that act on cytoplasmic membrane to facilitate the release of endolysins for bacterial lysis. By metagenomic approaches, we have identified two antibacterial proteins coding genes *tmp1* and *tmp3* with a high-level of similarity towards holin-like toxin genes. Functional characterization of Tmp1 and Tmp3 as recombinant proteins revealed the identified proteins to exhibit broad spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria including pathogenic clinical strains. By mutational analysis we found that, by modulating the average hydrophobicity, antibacterial activity could be enhanced. Fluorescent microscopic analysis confirmed the primary site of action of the identified proteins to be on cell membranes. Till date the 3-dimensional structure for holins or holin-like proteins has not been elucidated. By molecular modelling, a 3D structure for the identified proteins was modelled and validated. The stability of the predicted structure was assessed by molecular dynamics simulations in water and membrane environment and was found to be stable. CD spectroscopy and 2D TOCSY NMR experiments were performed to validate the modelled structure of Tmp3. The proposed structure of Tmp3 was in agreement with previously reported antibacterial proteins with broad spectrum activity. To our knowledge, this is the first report of holin-like proteins with broad spectrum antibacterial. Lead peptides identified in this study opens gateway for exploring the potential of holins and holin-like proteins as effective antibacterial agents.

Keywords Holins, antibacterial activity, molecular modelling, 3D structure

Structure of the bacteriophage T4 long tail fiber needle-shaped receptor-binding tip

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Bacteriophages are the most numerous organisms in the biosphere. They are exploited in an emergent array of applications including bactericidal and experimental phage therapy applications. In spite of their biological significance and the spectrum of potential applications, little high-resolution structural detail is available on their receptor-binding fibres. Here we present the crystal structure of the receptor-binding tip (D10 plus D11, Fig. 1) of the bacteriophage T4 long tail fibre, which is highly homologous to the tip of the bacteriophage lambda side tail fibres. It reveals an unusual elongated six-stranded anti-parallel beta-strand needle domain containing seven iron ions coordinated by histidine residues arranged co-linearly along the core of the biological unit (Fig. 2). At the end of the tip the three chains intertwine forming a broader head domain, which contains the putative receptor interaction site. The structure reveals a previously unknown beta-structured fibrous fold, explains the remarkable stability of the fibre and provides a framework for mutations to expand or modulate receptor-binding specificity.

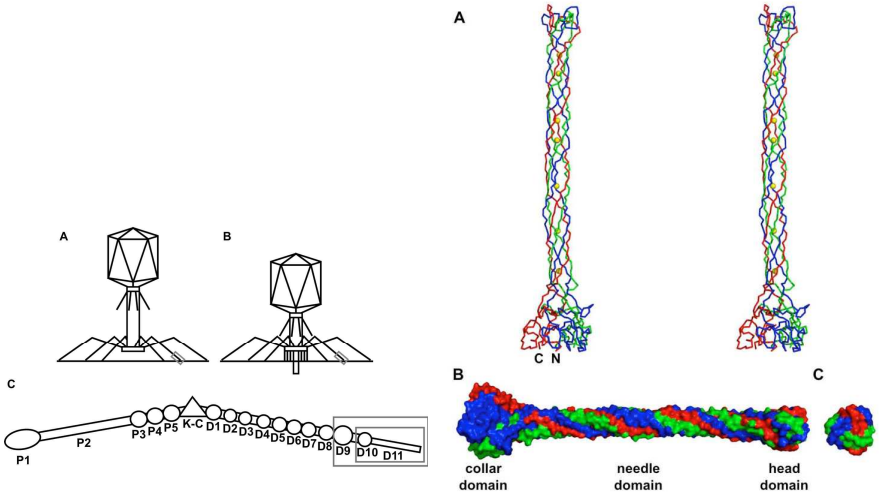

Fig. 1. Bacteriophage T4 and its long tail fibres. A-B. Schematic representations of bacteriophage T4 attached to a bacterial membrane before (left) and after (right) contraction of the outer tail tube. The tip domain of gp37 is boxed. C. Schematic representation of the bacteriophage T4 long tail fibre. Domains P1-5 correspond to gp34; the knee-cap domain (K-C) is formed by gp35, while the distal part of the fibre, consisting of gp36 and gp37, is divided into regions D1-11. The expressed protein, gp37(651-1026), corresponds to D9-11 (larger grey box), while the crystallised fragment, gp37(785-1026), corresponds to D10-11 (smaller grey box).

Fig. 2. Overview of the structure. A. Stereographic ribbon representation of gp37(785-1026). Chains A, B and C are coloured red, green and blue, respectively. The N- and C-termini of chain A are labelled. B-C. Surface representations of the structure of gp37(785-1026) seen from the side (B) and top (C) to illustrate the extensive intertwining of the three protein chains in the trimer.

Keywords fibrous fold; gene product 37 (gp37); iron binding; receptor binding; viral fibers; X-ray crystallography

Study on Antioxidant Mechanism of *Lactobacillus fermentum* I5007 for Pigs

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Three experiments were conducted to investigate the effects of *Lactobacillus fermentum* I5007 on the antioxidant mechanism for pigs *in vivo* and *in vitro*. In experiment 1, free radical-scavenging activities of *L. fermentum* I5007 were analyzed using free radical production systems *in vitro*. The results showed that *L. fermentum* I5007 possessed the ability to resist hydrogen peroxide. The scavenging capacities of *L. fermentum* I5007 against 1,1-diphenyl-2-picrylhydrazyl radical, hydroxyl radical, and superoxide anion radical increased as *L. fermentum* I5007 concentration increased. Experiment 2 was conducted to investigate the effects of *L. fermentum* I5007 on the redox state of healthy and oxidative-stressed weanling piglets on the base of establishing an oxidative stress model. An intraperitoneal injection of diquat resulted in the oxidative stress of weaned piglets. Oral administration of *L. fermentum* I5007 increased the growth performance of weaned piglets, improved the antioxidative defense system, and alleviated the oxidative damage caused by oxidative stress. Feeding experiment and slaughter experiment were conducted to investigate the effects of *L. fermentum* I5007 on growth performance, carcass traits and the redox state of growing-finishing pigs. The present study indicated that supplementation of *L. fermentum* I5007 increased the concentration of some unsaturated fatty acids in the LD muscle and increased antioxidative enzymes activities, and decreased the concentration of malondialdehyde without affecting growth performance or carcass traits.

In conclusion, *L. fermentum* I5007 developed its antioxidation by lowering the production of free radicals, increasing antioxidative enzymes activities and reducing the level of malondialdehyde.

Keywords: *Lactobacillus fermentum* I5007, Antioxidantative property, Oxidative stress, Growth performance, Carcass traits

Syntheses of new bacteriocins by different strains *Lactococcus lactis* subsp. *lactis* and their properties

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Lactococcus lactis subsp. *lactis* which belongs to group of lactic acid bacteria is a producer of diverse bacteriocins. Biosynthesis of bacteriocins is a heritable trait of microorganisms, manifesting itself as the ability of each strain to produce at least one specific antibiotic substance; certain bacterial cells are capable of synthesizing bacteriocins of several types. Screening of the effective bacteriocin-synthesizing strains of *L. lactis* was performed. Altogether 520 lactococcal strains were isolated from raw milk probes and dairy products from various climatic regions; some strains were obtained after mutagen treatment, other by protoplast fusion. Physiological and biochemical features of new strains were studied and compared to the nisin-producing strain *Lactococcus lactis* ssp. *lactis* MSU. Antimicrobial activity studies revealed differences between the strains against individual groups of test microorganisms; the activities of the strains were also distinct from Nisaplin (a commercial preparation of the bacteriocin nisin). It was demonstrated that the bacteriocins they produced varied in antagonistic activity. Some strains, isolated from milk and dairy products of Moscow region, were nisin-synthesizing (inhibit Gram-positive bacteria only), only nine of the isolated 94 strains and fusant strains expressed a broad spectrum of activity against potential food pathogens: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, *Proteus vulgaris, Salmonella gallinarum* and fungi of *Aspergillus*, *Fusarium, Penicillium*, *Rhodotorula* and *Candida* genera. This property is unique for isolated natural strains of *Lactococcus lactis* species. These strains had high antibiotic activity up 3600 to 5640 IU/ml respectively for natural and fusant strains, as compared with nisin. The differences in the antimicrobial spectrum of the strains are known to be due to structural diversity of the bacteriocin synthesized. Synthesis of bacteriocins as a protein proceeds concurrently with growth of producer. Strains of *L. lactis* subsp. *lactis* produced bacteriocins in parallel to growth with maximum of antibiotic activity at 10-12 h of cultivation. The synthesis was regulated by the components of fermentation medium, the content of inorganic phosphate, yeast autolysate (source of amine nitrogen), and changes in carbohydrates and amino acids. As result we have found optimal composition of fermentation media.

The antibiotic complex isolated from strains appeared as a mixture of biologically active components which differed from each other in mass, R_f values, chemical nature and biological properties. Analytical HPLC, TLC, FAB-MS and FD-MMS methods were performed to determine the structure of new complex. A highly active bacteriocins were isolated from these strains; one of its fractions were similar to nisin in its biological activity and physico-chemical properties, but another have not been described yet in the literature and appeared to be as new entity with no analogues among biologically active substances in database BNPD.

Keywords: *Lactococcus lactis* subsp. *lactis,* bacteriocins synthesis, physico-chemical properties.

The first report of isolation of an antibiotic producer bacterium from the petroleum sludge

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Today, the development of Multidrug-Resistant (MDR) bacteria is a pressing public health problem. Therefore, there is a critical need to continually develop new antibiotic compounds. Microorganism obtained from petroleum oil sludge may be an alternative source of new antibiotics. In this study, we first time attempted to assay bacterial strains isolated from petroleum oil sludge samples collected from the Abadan petroleum refinery in order to production of antibiotic. The sludge samples used in this study were collected from Abadan petroleum refinery (Southwest of Khuzestan province, Iran). Three random samples of the sludge were collected during 2 months. Initially, 100 g of sludge was transferred in to Erlenmeyer, and then 100 ml of distilled water was added and sterilized by autoclaving. A mineral medium composed of K_2HPO_4, KH_2PO_4, $CaSo_4.2H_2O$, $MgSO_4H_2O$, $FeSo_4.7H_2O$ and $(NH_4)_2So_4$ was prepared. Finally, 100 ml of the sludge extract was added to 1 L of mineral medium and autoclaved. This liquid medium was used for enrichment of bacteria in sludge. The solid medium was prepared by adding agar to liquid medium and used as primarily isolation medium. Isolation of bacteria was performed using serial dilution and pour plate techniques. For screening of the antibiotic producing bacteria, pure colonies of the bacterial isolates were transferred to Erlenmeyer flasks containing Nutrient Broth (NB) medium and were incubated at 37 ˚C on a rotatory shaker (140 rpm) to produce antibiotic compounds. After 72 h, liquid culture centrifuged at 13000 rpm for 10 minutes, and then supernatant was extracted by ethyl acetate. The antibacterial activity of the obtained raw extract was evaluated at 100 mg/ml concentration using disc diffusion method against human pathogenic bacteria including *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 12711), *Listeria monocytogenes* (ATCC 19112), *Escherichia coli* (ATCC 11303), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (ATCC 19430),MRSA (Methicillin Resistant *Staphylococcus aureus*) (clinical), *Staphylococcus epidermidis* (clinical), *Bacillus anthracis* (clinical), *Bacillus cereus* (clinical), *Bacillus pumilus* (clinical), *Listeria monocytogenes* (clinical), *Pseudomonas aeruginosa* (clinical), *Escherichia coli* (clinical), *Proteus mirabilis* (clinical), *Brucella melitensis* (clinical), *Salmonella typhi* (clinical) and *Klebsiella pneumoniae* (clinical). Synthetic antibiotic discs were used as control. A total of 13 bacterial isolates were obtained from petroleum sludge but among them only one dark green pigmented bacterium was exhibited the capability of producing antibiotic compounds. Biochemical diagnostic tests revealed that this bacterium belong to the genus *Pseudomonas* and named *Pseudomonas* sp. IR-01. The antibacterial compound produced by intended bacterium was effective against all of the tested gram positive bacteria while gram negative bacteria except *E. coli* and *K. Pneumonia* showed resistance to it. MRSA, *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 12711) and *E. coli* (ATCC 11303) were the most sensitivie strains. All tested bacteria were multi-drug resistance (MDR) strains. The obtained raw extract from *Pseudomonas* sp. IR-01 displayed remarkable antibacterial activity against MRSA and *E. coli* (ATCC 11303) compared with antibiotic standard discs (Fig. 1). On the basis of the obtained results, the antibiotic compound produced by *Pseudomonas* sp. IR-01 can gives hope for treatment of infections caused by MDR bacterial strains and also, the petroleum sludge can represent a relatively untapped resource for new drug development.

Keywords: MDR, Antibiotic, Petroleum sludge.

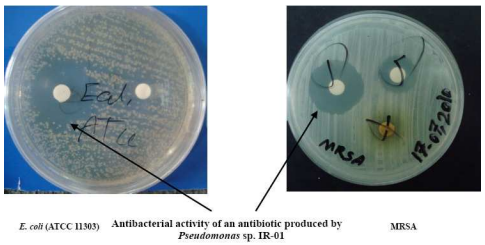

Figure 1: Antibacterial activity of ethyl acetate extract of *Pseudomonas* IR-01 against MRSA and E. *coli* (ATCC 11303)

The Inhibitor activity of *Lactobacillus rhamnosus* against rope-forming *Bacillus* strains

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In this study, it was investigated inhibitor activity of *Lactobacillus rhamnosus* used as biopreservative against rope-forming *Bacillus* strains as in vitro (agar diffusion method and in broth medium) and in vivo (in dough from bread flour).

Supernatants and cells of *Lactobacillus rhamnosus* were evaluated with regard to inhibitor activity after incubation for 24 hours. After incubation for 24 hours in broth medium, it was found that 6 isolate from 20 isolate (*Bacillus spp*.) were inhibited by both supernatants and cells. According to agar diffusion method, while 5 isolate are resistant to supernatant, 4 isolate are resistant to cells. Also in dough added only *Bacillus subtilis* ($1{,}3x10^4$ kob/g), counts of *Bacillus subtilis* increased, whereas an important change didn't occured in counts of *Bacillus subtilis* ($2{,}9x10^4$ kob/g) in dough added mix of *Bacillus subtilis* ($1x10^4$) and *Lactobacillus rhamnosus*. As a result, it was determined that while *Lactobacillus rhamnosus* has inhibitor activity against some *Bacillus subtilis* strains, it didn't have any inhibitor activity against some of them.

Keywords: *Lactobacillus rhamnosus,* ropy spoilage, dough

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The structure of the membrane-puncturing needle of the contractile tail bacteriophages P2 and ϕ92

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The needle or spike protruding from the center of the baseplate is a conserved feature found in all known phages with contractile tails. It has been proposed that this needle punctures the outer membrane of the cell during phage infection and tail contraction to create an opening for the tail tube [1]. The structure of one of these needles, the phage T4 gp5-gp27 complex, was reported earlier [1]. However, the structure of the membrane-interacting tip of the T4 needle is unknown because it is formed by a yet unidentified protein, which decorates the gp5 beta-helix [2].

We report here the crystal structure of the membrane-penetrating needle proteins of the well studied bacteriophage P2 and of the phage ϕ92, which infects polysialic acid-encapsulated *E.coli* K1 and K92. We characterize, for the first time, the atomic structure of the membrane-penetrating needle tip of two contractile tail bacteriophages. The two phages are genetically and morphologically unrelated, but their membrane-penetrating needles have a somewhat conserved structure, which also resembles T4 gp5, despite showing no significant sequence similarity. Gene product V (gpV) of bacteriophage P2 was identified as the needle (or spike) protein using antibody labeling earlier [3]. It has a glycine-rich C terminus containing the HxHxH motif. Only one protein out of about 300 ORFs of ϕ92 showed similar traits – gp138, which we assumed to be a structural homolog of P2 gpV. Both proteins are SDS- and urea-resistant trimers and are expressed in the soluble form in the cells.

The crystal structure of gpV shows that it is a long trimer and consists of two domains, the N-terminal OB-fold domain and the C-terminal triple-stranded beta-helical domain. The N-terminal half of gpV superimposes onto the N-terminal part of T4 gp5 very well despite exhibiting only 11% sequence identity, suggesting a common evolutionary origin for all of these proteins. The gpV and gp138 beta-helices are different from that of gp5 in that they taper strongly towards the C terminus and end in a very sharp tip (only 10 Å in diameter), making the whole protein look like a sharpened pencil, further supporting its membrane-penetrating function. The gpV and gp138 polypeptide chain topologies at the tapered tip are somewhat different and appear to be related by a chain swapping event, which occurred in the past.

The sharpened tip of gpV contains three ions – Fe, Ca and Cl – all positioned along the axis of the trimer, whereas gp138 contains only a Fe ion. In both proteins, the Fe ion is coordinated by six histidines (two from each of the three polypeptide chains of the trimer) from the conserved HxHxH motif.

In a parallel study, we have obtained the cryoEM structure of the bacteriophage ϕ92 baseplate. The cryoEM density confirms that gp138 and gpV form the central needle (or spike) of the baseplate and are likely function to create the initial opening in the membrane during tail contraction.

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Keywords: bacteriophage; membrane-binding protein; crystal structure; beta-helix

The use of *Lactobacillus brevis* PS1 to *in vitro* 1 inhibit the outgrowth of *Fusarium culmorum* and other common *Fusarium* species found on barley

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A total of 129 lactic acid bacteria (LAB) were screened for antifungal activity against common *Fusarium* spp. isolated from brewing barley. Four out of the five most inhibiting isolates were identified as *Lactobacillus brevis,* whereas one belonged to *Weissella cibaria. L. brevis* PS1, the isolate showing the largest inhibition spectrum, was selected and the influence of its freeze dried cell-free supernatant (cfsP) on germination of macroconidia as well as mycelia growth was investigated using *Fusarium culmorum* as target organism. Addition of cfsP into the growth medium at concentrations ≥ 2 % altered the growth morphology of *F. culmorum*, whereas at concentrations >5% the outgrowth of germ tubes from macroconidia was delayed and distorted. The presence of 10% cfsP completely inhibited the outgrowth of *F. culmorum* macroconidia. The activity of the compounds produced by *L. brevis* PS1 was higher at low pH values, i.e. pH < 5. Heating and/or proteolytic treatment reducedthe inhibitory activity of cfsP, indicating that *L. brevis* produces organic acids andproteinaceous compounds which are active against *Fusarium* spp.

Key words: antifungal, *Lactobacillus*, *Fusarium* spp., mycelia growth, macroconidia, germination, inhibition

Transcriptional response and antibiotic production by *P. fluorescens* Pf0-1 during competitive interactions with different bacterial species

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Most known soil bacterial species are organotrophs with considerable overlap in their metabolic abilities. Since carbon resources are limiting bacterial growth in most soils, competition between organotrophic bacterial species is to be expected. Interference competition, the active suppression of a competitor, is common among soil bacteria but the success depends on the properties of the competitor. Therefore, competitive strategies may need to be fine-tuned towards different competitors. This would need recognition of the competing species and, so far, it is no known if bacteria have this ability.

In the present study we describe transcriptional responses of the soil bacterial isolate *P. fluorescens* Pf0-1 (Class *Gamma-proteobacteria*) to three phylogeneticaly different bacterial competitors namely *Bacillus* sp. V102 (Class *Bacilli*), *Brevundimonas* sp. V52 (Class *Alpha-proteobacteria*), *Pedobacter* sp. V48 (Class *Sphingobacteria*). Each bacterial strain was grown on agar under carbon-limiting conditions in close proximity, but physically separated, from *P. fluorescence* Pf0-1. There was a strong impact of the identity of the competing bacterium on gene expression profiles of *P. fluorescens* Pf0-1. Only a small percentage of differentially expressed genes were common for all interactions. There was higher similarity in the gene expression response of *P. fluorescens* Pf0-1 to the Gram-negative bacteria as compared to the Gram-positive strain.

The expression of Pf0-1 genes related to signal-transduction mechanisms and secondary metabolite production was also strongly affected by the identity of the competing strain.

Gene expression data were complemented with the analysis of production of a broad-spectrum antibiotic. The broad-spectrum antibiotic was triggered during the interaction with the Gram-negative bacteria but not during the interaction with the Gram-positive *Bacillus* sp. V102.

Keywords: inter-specific competitive interactions; transcriptomics; antibiotic production

Ultrastructural study on the effect of an antibacterial compound produced by *Pseudoalteromonas piscicida* PG-02 against MRSA

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MRSA (Methicillin Resistant *Staphylococcus aureus*) is the most impotrant nosocomial pathogen which is not only resistant to Methicillin but also show resistance to many other antimicrobial agents; so, there is an urgent need to develop new antibiotics to deal with this bacterium. The pupose of this study was to understand the morphological changes in MRSA due to an antibacterial compound produced by *Pseudoalteromonas piscicida* PG-02, a marine bacterium isolated from a coastal sediment of the Persian Gulf. The tested marine bacterium has been produced an antibacterial compound with broad-spectrum antibacterial activity against both gram-positive and gram-negative bacteria. But its activity especially against MRSA was remarkable. Ultrastructral study on the effect of intended antibacterial compound on MRSA was done using Transmission Electron Microscope (TEM). At first, MIC and MBC values of the ethyl acetate extract of *Pseudoalteromonas Piscicida* PG-02 was determined against a MRSA strain using macrobraoth dilution method. Then, MRSA strain was treated with MIC concentration (40 mg/ml) and sub-MIC concentrations (37, 34 and 30 mg/ml) for 7 hrs. Sampling was done at 2, 4 and 7 hrs. Finally, all of the samples were mixed together and centrifuged at 4500 rpm, and then the cells were fixed in 4% glutaraldehyde. Later the cells were post-fixed in 1% osmium tetroxid. Washing was done by sodium cacodylate buffer and then the samples were dehydrated in gradualed gold ethanol series (30-100%). The dried cell blocks were the infiltrated by epoxy resin. Ultrathin sections were prepared using an ultramicrotome. Subsquently, the sections were stained by Uranyl acetate and lead citrate. For preparation of the control sample, MRSA cells were grown in MHB medium without extract. Ultimately, the ultrathin sections were analyzed using TEM (Philips, Netherland). TEM pictures showed that a disorganized cytoplasmic membrane was observed upon the extract treatment when compared to the smooth and intact cell membrane of the untreated bacterium (Fig. 1a and Fig. 2a). The membrane damage might be one of the causes for the death of the bacteria. Ultimately, ultrastructral study on the effect of antibacterial compound produced by *Pseudoalteromonas piscicida* PG-02 strain on MRSA compared with control revealed that the intended antibacterial compound affects the cytoplasmic membrane of MRSA; so, we can say that this antibiotic compound can be considered as a bactericidal agent against MRSA and further studies should be performed in order to purify and identify its chemical structure.

Keywords: TEM, antibiotic, ultrastructural study, *Pseudoalteromonas piscicida*, MRSA, Persian Gulf.

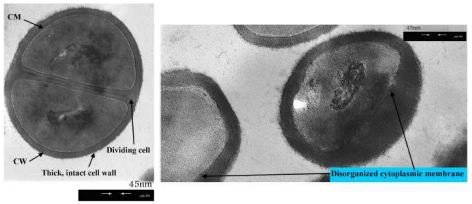

Figure 1: Transmision electron micrographs of MRSA. **a:** cell grown in the absence of antibiotic compound produced by *Pseudoalteromonas piscicida* PG-02, **b:** cells grown in presence of antibiotic compound produced by *Pseudoalteromonas piscicida* PG-02.

Uncovering the mechanism of resistance against type III bacteriocin, enterolysin A

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Enterolysin A (EnlA) is a peptidoglycan hydrolase produced by some enterococcal strains with a relatively broad inhibitory spectrum including enterococci, streptococci, pediococci, lactococci, and lactobacilli. This enzyme is being classified as a class III bacteriocin (large heat-labile bacteriocins) and it is interesting due to its domain structure resembling the structure of bacteriophage peptidoglycan-degrading enzymes (endolysins). N-terminal catalytic domain is responsible for cleaving chemical bonds in peptidoglycan and C-terminal domain has been thought to be responsible for EnlA binding to its cell wall substrate.

Using EnlA C-terminal domain fused with green fluorescent protein (GFP-CWB) followed by fluorescence assay we demonstrated that this part of EnlA represents true cell wall binding (CWB) domain. This domain is involved in specific recognition and binding of enterolysin A to the cell envelopes of sensitive cells. Any from bacterial strains resistant to EnlA displayed ability to bind GFP-CWT protein and at least some of resistant strains lack EnlA structural gene or adjacent genes of EnlA operon.

Although resistance to bacteriocins often results from the expression of so-called “immunity protein” (in bacteriocin producing strain encoded by the same operon as bacteriocin itself), we suppose that in the case of EnlA, resistance results from the absence of specific receptor(s) needed for EnlA binding.

Keywords enterolysin A; CWB domain; resistance

On detecting bacterial capsules under ambient conditions with nanometer resolution using AFM. Part I: AFM Imaging.

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The detection of bacteria extracellular polymers such as bacterial capsules/EPS is a critical issue in clinical microbiology since they play a prime role in pathogenesis and in the resistance to antimicrobials. They usually carry pathogenic antigens as virulence factors, contributing to cell attachment and evasion from host defenses.[1] Due to their extremely soft nature, they can easily be perturbed by common high resolution imaging techniques. For example, they remain poorly preserved in SEM imaging due to dehydration. Atomic Force Microscopy, a modern imaging technique capable of obtaining high-resolution images of even extremely soft surfaces under ambient conditions, has recently been suggested to be able to detect tiny amounts of extracellular substances such as capsules or EPS under ambient conditions, in some cases in strains where SEM/TEM did not them. [2] [3] [4] [5] This could especially be interesting for extremely thin capsules, which could remain elusive to optical microscopy or conventional staining methods. [6]

In line with those previous works, in this work, we report on the AFM detection of stable liquid-like structures around *S. epidermidis* cells belonging to three different strains: *S. epidermidis* ATCC 12228 (non-EPS producer), *S. epidermidis* ATCC 35983 (intermediate EPS producer) and *S. epidermidis* ATCC 35984 (high EPS producer). To this end, droplets of bacterial suspensions were deposited onto glass slides and left to dry under ambient conditions. The dried spot was extensively imaged by AFM, and three different regions could be distinguished: the centre, the periphery and intermediate areas. Interestingly, qualitatively the same results were obtained for the three studied strains. At the centre of the spot most of imaged bacteria appeared “naked”, showing no surrounding structures, or showing a small amount (see Fig.). While moving to intermediate areas between the centre and the periphery, bacteria usually appeared as embebbed in an amorphous liquid-like substance. These amorphous substances displayed a strong contrast in AFM phase images, revealing different physical or chemical properties compared to the bacteria. At the spot periphery, extensive amounts of those amorphous substances were detected. In the literature cited above, extracellular structures as those visible in the center/intermediate areas have been attributed to bacterial capsules. The first important observation is that the amount of liquid-like substance depends on where measurement is made on the macroscopic spot. This suggested that a kind of non-biological phenomenon was causing the appearance of those stable (evaporation-resistant) liquid-like structures. These findings are rationalised in the Part II of this communication.

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On detecting bacterial capsules under ambient conditions with nanometer resolution using AFM. Part II: Rationalising AFM findings.

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The results presented in the Part I of this communication, where extensive imaging covering different zones of the macroscopically heterogeneous system has been performed, suggest a physical or chemical origin of those substances, since the amount at which they are present just depend on where measurements are made in the spot. Also, the observation that cumuli of cells were completely embedded, sharing the same “capsular material”, as well as the enormous amounts (compared to the cell size) observed in areas at the periphery, suggested a possible non-biological origin of this material. Imaging of these liquid-like substances on a same location during several days did not show any appreciable change in their amount, suggesting that a rapid equilibrium was reached once the liquid was visually seen to macroscopically evaporate before the beginning of the imaging experiences. This certainly rules out these structures to be solely composed of water, as such ultrasmall volumes would become rapidly evaporated.

To shed light into the origin of these liquid-like structures, and specifically into the spatial variation observed, an XPS chemical analysis of the spots was performed. XPS spectra were recorded at equidistant points along a spot diameter. The distribution of the two most representative elements of the buffer, P and K, was mapped. The results showed a gradual increase in the surface concentration of these ions towards the periphery of the spot, thus matching the trend observed in the appearance of the liquid-like substances as shown by AFM imaging. This directly points to a close relationship between the surface concentration in buffer ions produced during evaporation and the formation of the liquid-like substances, again this fact suggesting a non-biological origin. KPi buffer is composed of an equimolar mixture of K_2HPO_4 and KH_2PO_4. Therefore, the relative atomic concentration between K and P is 3/2. By obtaining this relative abundance from the XPS survey spectra, it revealed to be very close to that stequiometric ratio, indicating that the detected K and P atoms came from the buffer used and were of non-biological origin. Taken together, the AFM and XPS data clearly revealed that the appearance of the extracellular liquid-like substances was related to the local concentration of the buffer ions. In order to test this hypothesis about the influence of buffer ions, the same AFM experiments were performed using distilled water instead of KPi buffer as suspending liquids. In those cases no evidence of extracellular liquid-like substances has been found when suspending liquid is water. Clearly, their origin must be linked to the ions present in the used buffers. To further clarify the origin of the liquid-like substances surrounding bacterial cells, and specifically, to find out if the bacteria could play some role in their appearance, structure and/or stability, experiments with non-biological particles (Al2O3 powder) were performed. At the center of the spot “naked” alumina particles were found. When moving towards the spot periphery, a behavior which is very similar to that encountered while imaging bacteria was found, with liquid-like substances detected around the alumina particles in this area. The results obtained with these experiments performed with non-biological particles definitively show that there is no need to invoke any biological structure or process for reproducing the phenomenon observed for bacteria.

We here propose deliquescence as the basic chemical property responsible of the appearance of the observed liquid-like substances. Deliquescence is a phenomenon by which certain substances are able to *absorb* moisture from the ambient *and* dissolve into them. It should not be mistaken with hygroscopicity, by which these substances adsorb or absorb moisture but do not form a solution upon absorption. Many of the salts used in common biological buffers are deliquescent. In the case of KPi, one of its component, K_2HPO_4, is highly deliquescent, while the other one, KH_2PO_4, is not.

This proposed explanation could also explain capsular or EPS structures visualized by other authors using buffers containing other deliquescent components such as $CaCl_2$ (one of the most deliquescent salts), or HEPES (with a deliquescent piperazine moiety).

Reduction in bacterial adhesion to a biocompatible 316LVM stainless steel after ionic implantation of silicon.

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Implant failure due to microbial colonization is an extremely important medical problem, which often leads to implant replacement. Modern surface modification techniques, such as ion implantation, can be explored to modify the surface of those materials so as to make them less “adhesive” towards microbes. In this work, Si+ ions have been incorporated into the surface of austenitic stainless steel 316 LVM and the effect of such modification on the biocompatibility and bacterial adhesion has been investigated *in vitro*. To this aim, human mesenchymal stem cells (hMSCs), as precursor of osteoblastic lineage, and bacterial strains relevant in infections related to orthopedic implants, i.e., *Staphylococcus aureus* and *Staphylococcus epidermidis*, have been assayed. In order to contribute to a meaningful understanding of the biological response of the modified material, changes introduced by the ion implantation procedure were evaluated in terms of the chemical surface composition, fine topography, surface Gibbs energy, isoelectric point and corrosion. Results showed that hMSCs attachment, spreading, viability and alkaline phosphatase activity were not affected by Si+ ion implantation, while the number of bacteria adhered diminished in static conditions. Also, the process reduced the bacterial adhesion rates and retention strength. Reduction in bacterial adhesion was higher for *S. epidermidis* than for *S. aureus*. This study shows that Si+ ion implantation is able to reduce bacterial adhesion to 316 LVM stainless steel surfaces without compromising its good biocompatibility.