ANTIBIOTIC ALTERNATIVES FOR THE NEW MILLENNIUM

ABSTRACT BOOK

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EuroSciCon

The inexorable rise in the incidence of antibiotic resistance in bacterial pathogens, coupled with the disappointingly low rate of emergence of new, clinically useful antibiotics, has refocused attention on finding alternatives to antibiotic treatments. This event will discuss the potential utility of phages, probiotics, antimicrobial peptides, herbal medicines and immune modulation for treating human and animal disease.

This event has CPD accreditation

Table of Contents

Day 1: Novel Alternatives to Antibiotics

Invited Speakers Abstracts

Novel Host Therapeutic Targets regulating M. tuberculosis infection

Mr Cornelis Korbee, PhD Student, Leiden University Medical Center, The Netherlands

The emergence of drug-resistant Mycobacterium tuberculosis (Mtb) strains and a concomitant decline in the number of newly approved antibiotic drugs poses a severe global health problem, prompting for complementing therapeutic approaches. Upon infection of macrophages, Mtb manipulates host signalling networks to block phagosome maturation and induce a niche for survival but knowledge of the molecular interactions at the pathogen-host interface that regulate this is scarce. To address this, we combined chemical genetics screening approach with transcriptomics and proteomics, revealing novel host therapeutic targets, signaling networks and mechanisms that regulate the intracellular survival of Mtb.

Pro- & Synbiotics - an alternative to antibiotics?

Professor Stig Bengmark, Honorary Visiting Professor, Division of Surgery & Interventional Science, University College London, UK

70% to 90% of all the IgA immunocytes of the body (plasma cells and plasmablasts) are produced and programmed in the large intestine. Western lifestyle is strongly associated with reduced and malfunctioning microbiota, dysbiosis, and associated with impaired immunity, descretely increased general inflammation, markedly reduced resistance to infections and development of chronic diseases. Reconditioning the gut reduces obesity, prevents chronic diseases and reduces rate and severity of infections. Supplementation of some specific pro- and synbiotic formulations such as Synbiotic 2000 are demonstrated to dramatically reduce rate of in infections in ICUs as shown particularly in liver transplantation and trauma.

Peptide antibiotics for nasal decolonisation of MRSA carriage

Mr Arif Felek, School of Biomedical & Healthcare Sciences, Plymouth University, Plymouth, UK

Epidermicin is a novel bacteriocin (bacterially derived antimicrobial peptide) with potent activity against Gram-positive bacteria, including MRSA. We have demonstrated that epidermicin has very low toxicity and high stability, it can protect Galleria mellonella wax moth larvae from MRSA infection and has equivalent activity to mupirocin in a murine model of nasal decolonisation. These properties justify further development of epidermicin towards Phase 1 trials.

Quorum sensing inhibitors as an alternate to conventional antibiotics

Dr. Pragasam Viswanathan, Associate Professor, VIT University, India

Antibiotic resistance is looming large in the horizon; antimicrobial agents with novel modes of actions (MOAs) are increasingly explored. Most efforts for the attenuation of bacterial virulence factors have focused on impeding with quorum sensing (QS), which is a mode of communication used for both within and between the bacterial species, in response to the cell-population density through the production and release of chemical signal molecules (auto-inducers) and regulates processes such as bioluminescence, virulence factors (VFs) expression, biofilm formation and etc. Our lab explores diverse strategies for controlling QS. Objectives include analyzing the anti-QS properties of various natural and synthetic compounds, which enable us to develop next generation antimicrobial agents, which includes the development of anti-QS agents-impregnated devices as a new schema of prevention for device associated infections.

Serendipity, bees, honey and wounds

Professor Jonathan Cooke, Royal Hampshire County Hospital, UK

Surgihoney is a novel sterile product which has been developed as a unique antimicrobial wound treatment. It consists of natural honey sourced from several sites, processed to produce different potencies of antimicrobial activity which greatly exceed other honey dressings by delivering free oxygen radicals over a prolonged period to colonised wounds. It is comparable to chemical antisepsis but appears to retain the wound healing properties of natural honey. The in vitro and clinical properties of Surgihoney will be discussed. This may be the first product that can deliver all the required roles in the healing process of wounds as well as being a potent and non-toxic antimicrobial active adainst multi-resistant microbes.

Antibiotic versus maggot therapy in diabetic foot ulcer

Professor Chumpon Wilasrusmee, Department of Surgery, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand

Our evidence suggests that the maggot wound therapy is significantly better in wound healing and cost-effectiveness than the conventional wound therapy e.g. shorter antibiotic time.

The potential use and delivery of bacteriophages for the treatment of Staphylococcus aureus wound biofilm infections

Diana Alves, Bath University, UK

Wound biofilms are major causes of impairment of wound healing and of patient morbidity. Current antibiotic options for a successful treatment are becoming scarce. This is the scenario seen for Staphylococcus aureus, a common cause of wound biofilms. Bacteriophages are being considered as a clever alternative to deal with bacterial infections. We are assessing the eradication effect of a phage cocktail on an established biofilm under a continuous flow. The phage cocktail stabilised in an oil-in-water nano-emulsion shows to be replicating causing a decrease of the biofilm over time. Simultaneously, we are addressing the delivery system by using a responsive dressing.

Bacteriophages for antistaphylococcal therapies - natural repertoire and limitations of choice

Malgorzata Lobocka, Institute of Biochemistry and Biophysics, PAS, Warsaw, Poland

Staphyloccus aureus is one of the major and most challenging causes of human and animal infections, due to the spread of antibiotic-resistant strains. Bacteriophages, which infect and kill antibiotic-resistant S. aureus strains are thus a promising alternative to control staphylococcal infections. The repertoire of naturally occuring staphylococcal bacteriophages warrants a wide range of phage preparations' strain specificity and their high lytic activity against bacteria grown in laboratory media. However, results of additional studies limit the subset of potential therapeutic phages to those that meet safety criteria and can be effective therapeutically under in vivo conditions.

Oral Presentation Abstracts

AN *IN VIVO* STUDY EXAMINING THE USE OF SILVER IONS, UVC AIR FILTRATION AND TIO2 PHOTOCATALYTIC PAINTS IN THE REDUCTION OF *MYCOPLASMA HYOPNEUMONIAE* IN THE ENVIRONMENT OF PIGS.

C, Browne., A, Loeffler., D.H, Lloyd., A, Nevel.

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As antibiotic resistance increases on a global level, agricultural practices involving antibiotic use are under increasing scrutiny. *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) is the causal agent of enzootic pneumonia (EP); a respiratory disease affecting pigs worldwide. As well as being a primary pathogen it also predisposes pigs to secondary bacterial or viral infections. Control of *M. hyopneumoniae* is difficult and relies on multiple strategies including vaccination, management practices and antibiotics. Vaccination reduces clinical signs and improves growths rates but does not prevent lung lesions or transmission. Management practices include reducing stocking density, however, the global demand for cheaper pork products is putting pressure on producers to increase intensity of pigs on units. Antibiotics are successful in controlling *M. hyopneumoniae* infections, but legislation means antibiotic usage on farms must be reduced. Additionally, *M. hyopneumoniae* have shown some resistance to the antibiotic tylosin. Alternate options are urgently needed to help control and prevent bacterial pathogens affecting farm animals.

In preliminary studies we demonstrated that silver ions, UVC air filtration and TiO2 photocatalytic paints (novel methods) had broad antimicrobial properties *in vitro*. (Browne, unpublished data) Silver ion solution at a concentration of 9ppm was able to inhibit growth of *M. hyopneumoniae*. TiO2 photocatalytic paints were successful in preventing growth after eight hours of exposure and UVC air filtration was most successful at killing 90% of *M. hyopneumoniae* after 7.59 seconds of exposure.

In the present study, these three methods were tested *in vivo.* Briefly, four groups of pigs (n=20) were inoculated with *M. hyopneumoniae* and allocated to four groups; positive control (PC), vaccination (V), a novel methods (NM) or a vac+novel group (VNM). Clinical signs (coughing), weight gain and pathological lesions at slaughter were recorded to assess the effects on the animal. Nasal swabs, environmental swabbing and air sampling were taken for culture and PCR recording differences in *M. hyopneumoniae* load between groups. Thirteen of the twenty pigs (61%) had lesions consistent with EP at slaughter. There were significant differences in the number of coughs (mean ± (SEM) between V – 0.08 ± (0.06) and NM groups – 1.79 ± (0.5) (P=0.0096). There were no differences observed in the weight gain of the pigs in any of the groups. At post mortem, EP like lung lesions were scored, there was an overall significant difference between all the groups (mean ± (SEM): PC– 9.75 ± (3.0), V – 0.2 ± (0.2) NM – 9.25 ± (3.2), VNM – 1.75 ± (1.4) (P=0.0160). Due to the fastidious nature of *M. hyopneumoniae* only one sample was positive by culture and confirmed as *M. hyopneumoniae* by PCR in the vaccination group. The number of positive PCR samples was similar in all of the groups, with no differences observed. General bacteriology (environmental swabbing and air samples) was also carried out but there were no significant differences seen in any of the groups.

The environmental devices did not influence infection, nor the amount of *M. hyopneumoniae* that was recovered from the environment. Further work is still needed to examine the use of alternative measures to reduce bacterial infections in farmed animals. This is the first report using silver ions, photocatalytic paints and UVC air filtration to inhibit *M. hyopneumoniae* growth both *in vitro* and *in vivo*.

SPECIFIC LYSIS OF STAPHYLOCOCCUS AUREUS BY THE BACTERIOPHAGE ENDOLYSIN STAPHEFEKT SA.100: IN VITRO STUDIES AND HUMAN CASE SERIES

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New strategies in the treatment of infections are warranted, as antibiotic resistance is emerging. Endolysins originating from bacteriophages combine two characteristics essential for such new strategies: powerful killing of bacteria and limited likelihood of emerging resistance.
The endolysin Staphefekt SA.100™ selectively targets *S. aureus*. It is the first registered endolysin and it has already been used by over a thousand patients on intact skin.
In vitro data from turbidity assays and bactericidal assays with clinical strains of methicillin susceptible (MSSA) and resistant (MRSA) *S. aureus* showed that lysis of *S. aureus* by Staphefekt is dose dependent, specific and efficient, compared to control strains of coagulase negative staphylococci. MSSA and MRSA proved to be equally susceptible to the endolysin, and MIC's did not differ between them, with a median MIC of 64 µg/ml in a specified setup.
No naturally occurring resistance against Staphefekt could be inferred from the MIC distribution amongst strains of *S. aureus*. Moreover, induction of resistance against Staphefekt could not be achieved *in vitro*. In contrast, resistance could be induced against another naturally occurring anti-staphylococcal enzyme (lysostaphine) and the frequently used antibiotic mupirocin. This supports the hypothesis that resistance against lytic myophage-derived endolysins is not likely to happen. The lytic activity seems such an essential part of the life cycle of the bacteriophages, that during over a billion years of co-evolution of bacteria and bacteriophages, natural selection has yielded endolysins that effectively target highly conserved essential structures in the bacterial cell wall that cannot easily be changed.

The *in vivo* effect of Staphefekt was evaluated retrospectively in two case series of *S. aureus* associated dermatitis. Of seven rosacea patients, three were lesional *S. aureus* carriers. After the local application of Staphefekt for one week, *S. aureus* was eradicated from the lesion in all positive rosacea patients, while other skin inhabitants remained present.

In another case series of eight patients, lesional *S. aureus* carriership, symptom relief and corticosteroid use were analysed after Staphefekt treatment. In six cases, *S. aureus* was found in skin cultures before treatment (three patients with constitutional eczema, two with contact dermatitis and one with peri-oral dermatitis). In 5 of 6 patients, symptoms diminished during treatment with Staphefekt, and patients reported less or no need of corticosteroids. In one patient with severe constitutional eczema, the burden of *S. aureus* carriership was very high and symptoms diminished only moderately, necessitating the use of corticosteroids and eventually cyclosporin. In the remaining two cases of eczema, no *S. aureus* was found. In one of them, Staphefekt had no effect on symptoms and corticosteroid use remained unchanged. In the other case, symptoms did diminish, but corticosteroid use was not completely abandoned.

The two case series provide evidence of the *in vivo* applicability of Staphefekt to specifically eradicate *S. aureus* without disturbing the normal skin flora. The reported corticosteroid use suggests that a quick relief of symptoms at the stage of local inflammation could best be achieved by combining symptomatic short term corticosteroid therapy with eradication of etiological *S. aureus* carriership by Staphefekt. These results support further clinical studies in a placebo controlled setting on the effect of Staphefekt on *S. aureus* related skin diseases.

ARE BIOCIDES EFFECTIVE AGAINST INFECTION MANAGEMENT OF DIABETIC FOOT ULCERS?

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The diabetic foot is one of the most important and costly complications of Diabetes *mellitus*. Although ischemic and neuropathic changes are of major importance for the initial pathophysiological role, it is the infection by multiresistant microorganisms and the local unfavorable microenvironmental conditions to the action of antibiotics that leads to infection chronicity and amputation. Therefore, new therapeutic protocols for the infection management of diabetic foot ulcers representing an alternative to antimicrobial compounds are urgent. Recently, a novel lytic bacteriophage cocktail with therapeutic potential against organisms causing diabetic foot infections was described[1]. Although inhibitory results from this cocktail are extremely promising, its broad application still depends upon clinical trials results. Meanwhile, available biocide compounds represent an important alternative for local antimicrobial therapy.

The aim of this study was to evaluate the inhibitory activity of available synthetic and natural biocides against *Staphylococcus aureus* (n=23, including 10 MRSA isolates) and *Pseudomonas* spp. (n=7) isolates obtained from diabetic foot ulcers, using the protocol described by Zainol et al. 2013[2]. Biocides tested included natural honey, a wound care product based on honey (L-mesitran®), iodopovidone and chlorhexidine, at the following concentrations: 80%, 70%, 50%, 20% and 15%. It is important to refer that L-mesitran® should be applied directly to the wound, without being diluted. However, in this study it was necessary to perform dilutions due to technical constraints.

Results showed that natural honey possessed the lowest inhibitory effect against the tested isolates. In fact, at an 80% concentration, it was able to inhibit the growth of 96.7% of the isolates, but with a bactericidal effect in only 16.7%. L-Mesitran® was able to inhibit all isolates at 80% and 70% concentrations, with a bactericidal effect of 80% and 60%, respectively. As this product includes 40% of medical grade honey, it can be concluded that its other components, such as propylene glycol, PEG 4000 and vitamins C and E, also contribute to its antimicrobial activity. Iodopovidone and chlorhexidine showed the highest inhibitory effect, as they were able to inhibit the growth of all isolates, with a bactericidal effect, at a 50% concentration.

In conclusion, all the synthetic biocides tested have a high antibacterial potential against diabetic foot ulcers isolates, representing an important alternative or a complement to antimicrobial therapy in ulcer infection management. Their efficacy should also be tested against biofilms structures, which are usually present in 60% of chronic wounds[3].

References:
[1]Mendes JJ et al. In vitro design of a novel lytic bacteriophage cocktail with therapeutic potential against organisms causing diabetic foot infections. J Med Microbiol 2014, 63:1055-1065.
[2]Zainol MI et al. Antibacterial activity of selected Malaysian honey. BMC Complementary and Alternative Medicine 2013, 13:129
[3]James JA et al. Biofilms in chronic wounds. Wound Repair Regen 2008, 16:37-44.

POTENTIAL OF ENTERIC BACTERIOCIN AS A NEXT-GENERATION ANTIBIOTIC WITH IN-CIRCULATION ACTIVITY

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A new strategy in the development of antibiotics against the threat of antibiotic resistance is to simply adopt defense strategies of bacteria themselves. Bacteriocins, antimicrobial peptides produced by bacteria, are an essential entity of such strategies. Colicins are channel-forming bacteriocins produced by *Escherichia coli*. They are bactericidal to other *E. coli* strains by forming a voltage-activated channel in the cell membrane.

Here we constructed a peptide consisting of a 28-residue antibody mimetic fused to the channel-forming domain of colicin Ia and named it pheromonicin. By altering native targeting of colicins, antibody mimetic targets fused colicin to form a lethal channel in the cell membrane to lead the leak/death of targeted pathogens.

Pheromonicins have nano-molar efficacy against thousand clinical isolates of antibiotic-resistant Gram-positive and -negative pathogens while they left enteric probiotic bacteria, *lactococcus*, *lactobacillus* and *Bifidobacterium* unaffected at the dosages that effectively killed pathogens. In thousand rodents, poultries and ungulates sepsis models, pheromonicin-treated animals survived administration of MRSA, or VRE, or MDR *P. aerugenosa*, or *P. anatipestifer* at doses that were lethal to controls.

With the inheritance of colicin's native advantages, nano-molar efficacy, the ability to act in the host circulation and easy genetically engineered, pheromonicin application cleared fatal bacterial infections *in vivo* that did not respond to currently employed antibiotics (vancomycin, amoxicillin, oxacillin, carbapenem etc). Pheromonicin may be of value as a novel antibiotic against MDR pathogens with targeted bactericidal and cytokine modulating activities.

VIBRIO CHOLERAE CULTURE SUPERNATANTS TO INHIBIT BIOFILMS PRODUCED BY GRAM POSITIVE PATHOGENIC BACTERIA

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Pathogenic bacterial species associated with infection disease and antibiotic resistance therapy are linked to biofilm formation. According to the Centers for Disease Control and Prevention, biofilm causes 65% of infection in developed countries. Competing infection of pathogenic by interfering via signaling molecules and surface active compounds could serve as an alternative treatment to conventional strategy. The objectives of this study were to screen and characterize the bioactive component of *Vibrio cholerae* culture supernatants against biofilm formed by gram positive pathogenic bacteria.

Gram positive pathogenic bacteria biofilms were challenged with twenty-one *V. cholerae* culture supernatants. Four culture supernatants (G1.2, A50.2, V15A, and B3.2) performed antibiofilm activity to inhibit more than 25% biofilm formation at 20% (v/v) concentration. The antibiofilm activity was effective to at least to one of gram positive pathogenic bacteria used in this study. Further characterization showed that supernatants of G1.2 and A50.2 isolate had polysaccharide and nucleic acid as the bioactive compounds, whereas V15A and B3.2 isolates had nucleic acid and protein as a dominant of bioactive compounds. This study clearly marks that bioactive compound from *V. cholerae* culture supernatants could potentially provide a promising source for developing biofilm inhibitors against pathogenic bacteria.

USEFULNESS OF CLASSICAL HOMEOPATHY FOR THE PREVENTION OF URINARY TRACT INFECTIONS IN PATIENTS WITH NEUROGENIC BLADDER DYSFUNCTION: A CASE SERIES

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Introduction: In patients with neurogenic bladder dysfunction due tospinal cord injury (SCI), especially recurrent urinary tract infections (UTI) are a frequent clinical problem. Often, conventional preventive measures are not successful.
Aims: to present the initial results of classic homeopathic treatment in addition to standard urologic care in these patients.
Setting and Design: descriptive case series
Methods and Material: after exclusion of morphologic abnormalities and initiation of a standard regime for prophylaxis, all patients with a neurogenic lower urinary tract dysfunction (NLUTD) due to SCI with more than 3 symptomatic UTI/year were offered additional homeopathic care. Symptoms were fever, incontinence, increased spasticity, decreased bladder capacity or pain/decreased general health combined with significant bacteriuria.
Results: Eight of ten patients whom the treatment was offered opted for homeopathic treatment. Morphologic and functional reasons for recurrent UTI were excluded by sonography, cystoscopy and urodynamics. After a median follow-up of 15 months, five patients remained free of UTI, whereas UTI frequency was reduced in three patients.
Conclusion: Our initial experience with homeopathic prevention of UTI in addition to standard urologic prophylactic measures is encouraging. Keys for a fruitful cooperation are well-qualified partners, mutual respect and the motivation to cooperate closely. For an evidence-based evaluation of this concept, prospective studies are required.

ISOLATION AND CHARACTERIZATION OF WALDIOMYCIN AS A NEW ANTIBIOTIC TARGETING BACTERIAL HISTIDINE KINASES

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The WalK/WalR two-component signal transduction system is essential for growth and is specific to low GC-content Gram-positive bacteria such as Bacillus subtilis, Staphylococcus aureus and Enterococcus faecalis. WalK is a membrane-linked histidine kinase (HK) composed of two cytoplasmic domains, a catalytic (kinase or ATP-binding) domain and a dimerization (DHp) domain containing a conserved phosphorylated histidine residue. We previously isolated a new antibiotic, waldiomycin as WalK inhibitor. In this paper, we will present how waldiomycin targets the common DHp domain to inhibit HKs including WalK. These results suggest that DHp domains can be an effective target for developing the antibiotics against bacterial signal transduction pathway.

THE ENHANCED FUNCTIONAL PROPERTIES OF FERMENTED CITRUS MAXIMA JUICE FOR THE MANAGEMENT OF ESCHERICHIA COLI O157:H7 INFECTION

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The advantages of phenolic phytochemicals for the management of chronic degenerative and infectious diseases have been widely reported. *Citrus maxima* fruit contains phenolic bioactive compounds with several functional properties, such as high antioxidant, antimicrobial and anticancer activities, lowering cholesterol level and preventing cardiovascular diseases. The application of lactic acid fermentation on *C. maxima* juice may play an important role to maintain and enhance the functionality of phenolic bioactives in a beverage system, particularly due to the activity of the beneficial probiotic bacteria. The objectives of this study were to perform

fermentation of two Indonesian local cultivars of whole *C. maxima* fruit juice using a probiotic strain of *Lactobacillus acidophilus* ATCC 4356 and investigate the antioxidant and antimicrobial properties against *Escherichia coli* O157:H7.

The *C. maxima* fruit juice of Nias and Madu cultivars at naturally acidic and neutral pH treatments were fermented for 48 h. The assays consisted of the total count of *L. acidophilus*, lactic acid content, glucose content, total soluble phenolic content, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, broth dilution assay for determining the antimicrobial activity, and the sensory evaluation. The optimum growth of *L. acidophilus* in Nias and Madu cv. at both pH treatments were achieved at 7 log CFU/mL at 18 h of fermentation. The success of such fermentation was also indicated by the decreasing amount of glucose content and increasing amount of lactic acid content in both *C. maxima* cultivars. A stable and high antioxidant activity (63-76%) was reached when the total soluble phenolic content fluctuated during 48 h of fermentation, indicating the possible synergistic effect of the phenolic bioactives from the fermented *C. maxima* juice. Fermentation using a probiotic strain of *L. acidophilus* could increase the fruit juice inhibition activity against *E. coli* O157:H7 until 88% at 18 h of fermentation and remained constantly high until 48 h of fermentation. The inhibition activity of the diluted fermented juice (1:2 and 1:5 dilution) increased after 48 h of fermentation. At 18 h fermentation time, the optimum growth of *L. acidophilus* and the highest inhibition activity of *E. coli* O157:H7 were achieved although the concentration of lactic acid known for its antimicrobial properties was low. These result suggested the potential role of other compounds, such as the mobilized phenolic bioactives as a result of probiotic fermentation, for the enhanced antioxidant and antimicrobial activities of *E. coli* O157:H7. The sensory evaluation showed that the fermented *C. maxima* juice of both cultivars was accepted by the semi-trained panelist and the Madu cv. had the highest score for the overall attributes, i.e. 3.5 out of 5.

ACTINOMYCETES FROM MARINE SEDIMENT SHOWED ANTIBIOFILM ACTIVITY AGAINST SEVERAL PATHOGENIC BACTERIA

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Pathogen within biofilm induce chronic infection due to the resistance to conventional antibiotics treatment and host immune response compared to planktonic bacterial. Nowadays, there are some Actinomycetes species had been known for their potential bioactive compound against biofilm. This research was aim to screen the multispecies antibiofilm activity from Indonesian marine sediment Actinomycetes extracts in vitro against pathogenic Gram negative bacteria.
The result demonstrated that isolates KP 12 and CW 17 had the highest activity to inhibit the biofilm. *Pseudomonas aeruginosa* biofilm were inhibited by KP 12 isolate, Enterotoxigenic *Escherichia coli* (ETEC) by KP 04 isolate, and *Vibrio parahaemolyticus* were inhibited by bioactive compound from CW 17 at 5% . Supernatants of KP 12, CW 17, and KP 04 isolate had polysaccharide as the bioactive compound, whereas SW 13 supernatants had protein as the bioactive compound. In the destruction assay, both *P.aeruginosa* and *V.parahaemolyticus* mature biofilm were greatly destructed by SW 03 isolate and CW 01 isolate. Molecular identification of the isolates performed that isolates KP 12, CW 17, KP04, SW 13 showed similarity with *Streptomyces sp.* and CW 01 showed similarity with *Arthrobacter sp.* Research on characterization and purification of the bioactive compound from actinomycetes are important as an alternative strategy to overcome bacterial infections.

DEVELOPMENT OF PHARMABIOTICS AS ANTIBIOTIC ALTERNATIVES FOR SEAFOOD SECURITY AND MARINE AQUACULTURE HEALTH: TWO CASES OF STUDY IN VIETNAM

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Pharmabiotics are any biological materials of gut microbes, including probiotics, bacteriocins, bacteriophages and bioactive molecules. They have been introduced in food, agriculture and clinical settings to replace the use of traditional antibiotics, which led to current threat posed by multi-drug resistant bacteria. This research aims to develop bacteriocins or bacteriocin-producing bacteria as new pharmabiotics for improving seafood security and marine aquaculture health. We screened bacteria isolated from Vietnamese traditional fermented foods and marine animals of interest to the aquaculture industry (lobster, tiger shrimp, otter clam, snubnose pompano and cobia) for antimicrobial and bacteriocin-like activities in order to uncover biodiversity of bacteriocin producers, and explore the potential application in seafood preservation and marine aquaculture. In total, 32 screened isolates showed antimicrobial activities and 15 of these exerted bacteriocin-like activities. Sequencing of 16S rRNA genes identified the isolates as members of the nine genera *Lactobacillus, Bacillus, Proteus*, *Providencia*, *Klebsiella*, *Alcaligenes*, *Enterococcus, Enterobacter* and *Cronobacter.* In some cases, further analysis of housekeeping genes, *rpoB* for *Proteus* and *recA* for *Klebsiella,* as well as biochemical tests was necessary for identification to species level, and some of the *Proteus* isolates may represent novel species. The bacteriocinogenic isolates showed a wide antimicrobial spectrum against foodborne and animal pathogens, which opens the way to their potential use as marine drugs and probiotics in food, aquaculture, livestock and clinical settings. As the first case of study, two strains *Lactobacillus plantarum* T8 and T13 were found to produce bacteriocins Class I (Lantibiotics), which remained active at 121°C for 15 min, at pH 4-10 and with proteinase K but deactivated by α-chymotrypsin treatment. The application of culture extract from the strain T13 with cell concentration of 10^{10} CFU/ml or crude bacteriocin extract from the strain T8 with bacteriocin activity of 800 AU/ml was shown to prolong the chilling preservation of fresh cobia meat compared to control within first 7 days. As the second case of study, the protective effect of bacteriocinogenic *Bacillus* and *Lactobacillus* isolates were tested in aquaculture-raised spiny lobster (*Panulirus ornatus*) juveniles. Lobsters in the probiotic treatments displayed increased growth and reduced feed conversion rates after 60 days, and increased survival rate after pathogen *Vibrio owensii* DY05 challenge relative to the control. This study represents the first evidence of the use of bacteriocins or bacteriocin producers as biopreservatives for fresh cobia meat and as probiotics for lobsters.

ARTILYSIN ART-175: EFFECTIVE KILLING OF PERSISTENT AND MULTIDRUG-RESISTANT STRAINS PERSISTERS OF PSEUDOMONAS AERUGINOSA COMBINED WITH A LOW RISK OF RESISTANCE

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Multidrug-resistant Gram negative bacterial pathogens and especially P. aeruginosa form an increasing global threat. Persistent bacteria are being responsible for re-occuring infections.
Due to the growing resistance against classical antibiotics, there is a clear need for molecules with a new mode of action. Artilysins constitute a novel class of efficient enzyme-based antibacterials. They are recombinant fusion proteins consisting of a bacteriophage-encoded endolysin, which degrades the peptidoglycan, combined with a targeting peptide that transfers the endolysin through the outer membrane of Gram-negative bacteria.

In contrast to the basic endolysin, Art-085 and Art-175 pass the outer membrane and kill Pseudomonas aeruginosa, including multidrug-resistant strains, in a rapid and efficient (~5 log) manner.
Time-lapse microscopy confirms that Art-175 punctures the peptidoglycan layer within a minute, inducing a bulging membrane and complete lysis. Minimal inhibitory concentration (MIC) experiments show Art-175 to be highly effective on *P. aeruginosa.* The MIC_{90} of 10µg/ml was independent of the strains being highly resistant to antibiotics. Resistance development against Art-085 and Art-175 is not observed within 20 experimental cycles on all strains investigated. Whereas a significant resistance development against ciprofloxacin occurred already within 7 cycles of the MIC experiments. The strains PAO1p and Br257 showed a significant resistance development against ciprofloxacin with a more than 30-fold increase on Br257.
As Art-175 does not require an active bacterial metabolism for its antibacterial activity, thus it has a superior bactericidal effect against P. aeruginosa persisters (up to more than 4 log).
In summary, Art-175 is a novel antibacterial, well suited for a broad range of applications and with the unique potential to target persister-driven chronic infections.

ANTIBACTERIAL MODE OF ACTION OF CHICKEN CATHELICIDIN-2

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The increasing numbers of antibiotic resistance and the continuously declining approval of new antibiotics represent a serious threat to public and veterinary health. Antimicrobial peptides are considered an alternative to antibiotics with potential use in human and veterinary medicine. In this study the mechanism of antimicrobial killing of chicken cathelicidin-2 (CATH-2) against Gram-positive and Gram-negative bacteria was investigated. Colony count assays revealed MIC values of 1.25 to 5 µM for E. coli and MRSA, but membrane potential assays with DiSC3(5) showed that already at very low concentrations (0.19 µM), CATH-2 instantly perturbs the membrane potential of MRSA. Live-imaging with confocal fluorescence microscopy was performed, which demonstrated that FITC labeled CATH-2 mainly localized on the membrane of E. coli, with a preference for the bacterial septum of dividing cells. Upon binding the bacterial membrane was readily permeabilized as shown by propidium iodide influx into the cell. To further clarify the mechanism of bactericidal action of CATH-2, E. coli and MRSA were examined by transmission electron microscopy. Compared to untreated bacteria, E. coli and MRSA incubated in the presence of CATH-2 showed dose-dependent morphological changes. At sub-MIC concentrations of CATH-2, intracellular granulation and some cytoplasmic retraction were observed, while at MIC values and higher, membrane breakage and cell lysis occurred. These results show that, although the bacterial membrane is the initial target of CATH-2, intracellular effects should not be excluded from the antimicrobial killing mechanism of CATH-2.

EFFICACY OF LIPOSOME-ENCAPSULATED CIPROFLOXACIN AGAINST *BURKHOLDERIA PSEUDOMALLEI* STRAIN K96243

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Liposome-encapsulated ciprofloxacin for inhalation (CFI) was investigated as a putative post-exposure therapeutic agent against *Burkholderia pseudomallei,* the causative agent of the disease melioidosis. This organism has a low infectious dose by the aerosol route, is inherently antibiotic resistant and is classed by the US Center's for Disease Control as a Category B agent of concern.
The efficacy of CFI against *B. pseudomallei* strain K96243 was compared to non-encapsulated ciprofloxacin *in vitro*, using intracellular J774A.1 macrophage assays, and *in vivo* using a mouse model of *B. pseudomallei* infection. Mice were challenged by the intranasal route and treated 6 hours post challenge with 50 mg/kg CFI via intranasal instillation to the lung once a day, or given 50 mg/kg oral ciprofloxacin delivered twice a day, and monitored for 60 days.

The *in vitro* assays showed no difference between CFI and ciprofloxacin in reducing bacterial load in macrophages. However, in murine studies, CFI delivered to the lung by intranasal instillation for 3 or 7 days, provided a significant level of protection ($P<0.05$) (when compared to orally delivered ciprofloxacin), against a lethal *B. pseudomallei* challenge. Specifically, six out of 10 mice survived when therapy was administered for 3 or 7 days.

In this study, intranasally delivered CFI was superior to oral ciprofloxacin in protecting against *B. pseudomallei*. Consequently, inhaled CFI may be a promising medical countermeasure for use in the event of a deliberate release or in natural cases of *B. pseudomallei.*

FLUORESCENT IRON CHELATORS: ANTIMYCOBACTERIAL ACTIVITY AND DRUG-MEMBRANES INTERACTION STUDIES IN LIPOSOMES AND MACROPHAGES

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Bacterial resistance to currently used antibiotics is still one of the most important problems for human health and the development of new antimicrobial therapies remains crucial. The process of iron acquisition represents a pathway which can be successfully targeted by novel therapeutic tools and iron restriction has been shown to improve the outcome of a number of infectious diseases.

Our group has been studying the use of rhodamine-derived iron chelators to control the intramacrophagic growth of *Mycobacterium avium*. Previous results showed that the chelation of iron is a determinant but not sufficient property for the antimicrobial activity of these new molecules. Instead, biological activity seems to be dependent also on the nature of the fluorophore structure, since the rhodamine B isothiocyanate derived ligands (MRH7, MRB7) are more effective than those derived from carboxy-rhodamine (MRH8, MRB8) [1].Since these results suggest that the lipophilicity of the chelators and their ability to interact with membranes are crucial for their final biological effect, drug-membrane interaction studies were performed to understand the mechanism of drug action and allow us to develop antibiotics with higher affinity for their targets.

Herein we report the results obtained for the study of the interaction of iron chelators with membrane models, using distinctive techniques, as well as distribution studies within the macrophages.

The results obtained revealed that chelators conjugated with the same fluorophore show very similar behaviour in the presence of liposomes and provided evidence that the more active compounds strongly interact with the lipid phase while the less-active do not. Confocal microscopy studies show that the distribution in macrophages is similar for all tested chelators but the fluorescent signal inside macrophages is lower for MRH8 and MRB8 and this is probably justified by their higher water solubility and consequently lower ability to cross cell membranes in opposition with MRH7 and MRB7.

New chelators, MRH10, MRH9 and MRB9, were recently synthetized in order to get insight on the functional groups in the structure of the chelators that are determinant for the antimycobacterial effect.

Finally, we expect to be able to correlate the different affinity of each chelator for lipidic phases and the distribution inside the cells with the chelators ability to interact with cell membranes and compartments that should be crucial to reach the infection target and include this knowledge in the future design of iron chelators and improve their biological activity.

[1] Moniz, T., et al.,. Journal of Inorganic Biochemistry, 2013, **121**, p. 156-166.

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EVALUATION OF THE ANTIBACTERIAL ACTIVITIES OF SOME SELECTED EGYPTIAN HONEYS

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Abstract:
In this study, three commercially available Egyptian honeys from different floral sources (citrus, clover, and marjoram) were investigated for antibacterial activities against Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 8739, and Pseudomonas aeruginosa ATCC 27853, using a broth micro-dilution method. All the honeys tested showed to be free from starch, beet sugar, and reducing sugars, ruling out the possibility of adulteration. Marjoram honey exhibited the lowest minimum inhibitory concentrations (MIC) and the lowest minimum bactericidal concentrations (MBC) against the three tested organisms (MIC of 25% w/v and MBC of 50% w/v) except in the case of S. aureus ATCC 6538, in which an MBC value of 90% w/v was observed. Similarly, clover and citrus honeys showed equivalent activities against P. aeruginosa ATCC 27853 and S. aureus ATCC 6538, respectively, with MIC values of 25% w/v and MBC values of 50% w/v. Overall, against all the microorganisms tested, the three types of honey were more effective than an artificial honey solution (having an MIC of 65% and an MBC of 100%), which were also supported by the electron microscopy analysis, indicating that factors other than osmolarity are responsible for the antibacterial activities of the honeys under investigation. The observed decrease in the antimicrobial activity of clover and marjoram honeys after addition of catalase indicated that their antibacterial effect could be attributed, at least partially, to their peroxide action. In contrast, citrus honey showed non-peroxide antimicrobial activities against all the test organisms except E. coli ATCC 8739. Based on the results of this study, the tested Egyptian honeys may have the potential to be utilized as promising antimicrobial agents for prevention and treatment of bacterial infections. Further studies are required to elucidate other possible antimicrobial mechanisms of the tested honeys as well as their influence on the expression of virulence factors of the tested pathogens.

Keywords:
Egyptian honey, Citrus honey, Clover honey, Marjoram honey, Antibacterial activity; Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC), Non-peroxide activity.

Poster Presentation Abstracts

INHIBITION OF STAPHYLOCOCCUS AUREUS INVASION INTO BOVINE MAMMARY EPITHELIAL CELLS BY CONTACT WITH LIVE LACTOBACILLUS CASEI

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Staphylococcus aureus is one of the main pathogens involved in ruminant mastitis. Severity of bovine S. aureus mastitis is highly variable, from subclinical to gangrenous mastitis. Nevertheless S. aureus factors that may explain this variability are still poorly documented. There is a great demand for the reduction of antibiotics use in veterinary medicine. Besides, these antibiotics have a low efficiency against staphylococcal mastitis. There is thus a need to develop alternative strategies. One feature of S. aureus is its ability to persist within the mammary tissue and induce chronicity of the infection. One of the main causes is the S. aureus ability to adhere to and internalize into host cells. The concept of biological control, using natural inhibitory properties of lactic acid bacteria (LAB) as probiotics, is now well established in humans and animals and is used to reduce, prevent or treat bacterial infections. This work aims to better characterize the mammary microbiota in particular its LAB population, which might exert a positive effect on host health, and to determine its potential to modulate adhesion and internalization of S. aureus. This work was realized using two S. aureus strains as representatives of strains inducing severe (S. aureus RF122) or mild and chronic (Newbould 305) mastitis. Our results showed that the cellular invasion capacities seems to be key element in the chronicity observed in Newbould 305 mastitis. This was determined by genomic, proteomic and phenotypic comparison with RF122. Besides, we showed that the bovine mammary ecosystem presents a LAB biodiversity and that these LAB presented interesting probiotic capacities. This was shown in an in vitro model of tripartite interaction, between S. aureus, Lactobacillus casei and the mammary epithelial cells MAC-T. In this system, L. casei impairs S. aureus adhesion and internalization without modifying the viability and the morphology of cells. The inhibition depends on a direct contact with live L. casei. Identification of the molecular basis of this inhibition is currently under investigation. These results open new perspectives in the application of lactic acid bacteria as mammary probiotics.

Key-words: Mastitis, Staphylococcus aureus, Lactobacillus casei, Adhesion, Internalization, MAC-T

Reference ID:
Damien S. Bouchard Lucie Rault, Nadia Berkova, Yves Le Loir and Sergine Even. Inhibition of Staphylococcus aureus invasion into bovine mammary epithelial cells by contact with live Lactobacillus casei. Appl Environ Microbiol. 2013 Feb;79(3):877-85. doi: 10.1128/AEM.03323-12.

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF NEWLY SYNTHESISED S-(2,3,4,5,6-PENTABROMOBENZYL)ISOTHIOUREA DERIVATIVES

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Background:
One of the main reasons for therapeutic failures in treatment of bacterial infections is the microorganisms resistance, especially hospital strains, to a broad range of antibiotics. Therefore it is necessary to search for new compounds active against microorganisms. Izothiourea derivatives are interesting group of synthetic agents. Our previous study indicated the activity of S-(2,3,4,5,6-pentabromobenzyl)isothiourea against bacteria and fungi strains. This agent was the parent compound of the newly synthesised 7 substances analysed during this research.
Methods:
In this study, we determined in vitro the antimicrobial activity of 7 newly synthesised S-(2,3,4,5,6-pentabromobenzyl)isothiourea derivatives and their parent compound. The antibacterial and antifungal activity was examined by the disc-diffusion test and the MIC values determination method according to CLSI recommendation. The studied compounds were dissolved in DMSO. 0.4 mg of a new isothiourea derivatives were applied onto paper discs. For MIC determinations ± efflux pump inhibitor (PAbN - 0.08 mg/L), concentration of tested compounds in solid medium ranged from 0.39 to 400 mg/L. The reference ATCC strains of Gram-positive bacteria, non-fermentative Gram-negative rods, *Enterobacteriaceae* family and yeasts as well as clinical strains of *S. aureus* MRSA and *S. epidermidis* MRSE were applied.
Results:
In general, all studied new agents (except one - S-(2,3,4,5,6-pentabromobenzyl)-N-fenylisothiourea hydrobromide) were active especially against Gram-positive cocci:

Staphylococcus aureus (including MRSA), *S. epidermidis* (including MRSE) as well as *Bacillus subtilis and Geobacillus stearothermophilus* - MICs ≥3.125 mg/L. *Enterococcus* spp. were also sensitive to these derivatives - MICs ≥12.5 mg/L. Moreover, the parent compound and its 3 derivatives: S-(2,3,4,5,6-pentabromobenzyl)-N-methylisothiourea hydrobromide, S-(2,3,4,5,6-pentabromobenzyl)-N-ethylisothiourea hydrobromide and S-(2,3,4,5,6-pentabromobenzyl)-N,N,N,N-tetramethylisothiourea hydrobromide, exhibited also activity against non-fermentative Gram-negative rods and bacteria from *Enterobacteriaceae* family – MICs ≥25 mg/L. These 6 derivatives active against bacteria and the parent compound were substrates of the efflux pumps occurring in *P. aeruginosa, S. maltophilia, A. baumannii E. coli and E. cloacae* strains. On the other hand all studied new agents (except one - S-(2,3,4,5,6-pentabromobenzyl)-N-fenylisothiourea hydrobromide) were active against fungi (diameter of zone of inhibition 11-27 mm).
Conclusions:
Presented data indicated the structure-activity relationships among this class of agents. Obtained results give the reasons for searching for active substances to microorganisms among new S-(2,3,4,5,6-pentabromobenzyl)isothiourea derivatives.

The study was supported by the Foundation for the Development of Diagnostics and Therapy, Warsaw, Poland.

IN VITRO ACTIVITY OF NEWLY SYNTHESISED S-(3,4-DICHLOROBENZYL)ISOTHIOUREA DERIVATIVES AGAINST FUNGI AND AGAINST BACTERIA, INCLUDING STRAINS POSSESSING MDR EFFLUX SYSTEMS

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Background:
Isothiourea derivatives are interesting group of synthetic agents. Their diverse biological activity and clinical application is widely known. Recently, more attention was given to the antibacterial activity of new S-(benzyl)isothiourea derivatives. The aim of this study was to investigate the antimicrobial activity of nine new N-substituted S-(3,4-dichlorobenzyl) isothiourea compounds. The previous study indicated the activity of S-(3,4-dichlorobenzyl) isothiourea against *S. epidermidis, E. coli, K. pneumoniae* and *S. maltophilia* strains. Moreover, in the current study, the investigation whether these derivatives are the substrates of the MDR efflux pumps was undertaken. The efflux pumps from RND family in Gram-negative rods remove various groups of compounds including antibiotics, from bacterial cells.
Methods:
In this study, we determined in vitro the antimicrobial activity of 9 newly synthesised S-(3,4-dichlorobenzyl)isothiourea derivatives and their parent compound. The antibacterial and antifungal activity was examined by the disc-diffusion test and the MIC and MBC values determination methods according to CLSI recommendation. 0.4 mg of a new isothiourea derivatives were applied onto paper discs. During MIC values determinations ± efflux pump inhibitor (PAbN - 0.08 mg/L), the concentration of tested compounds in broth medium ranged from 0.39 to 400 mg/L. The reference ATCC strains of Gram-positive bacteria, non-fermentative Gram-negative rods, *Enterobacteriaceae* family and yeasts as well as clinical strains of S. *aureus* MRSA and S. *epidermidis* MRSE were applied.
Results:
The parent compound S-(3,4-dichlorobenzyl)isothiourea was active against Gram-positive cocci: *Staphylococcus* sp. (including MRSA and MRSE) and *Enterococcus sp.* (MICs 50-200 mg/L) as well as against Gram-negative rods (MICs 6.25-200 mg/L), especially against *P. aeruginosa* and *S. malthophilia* (MICs 6.25-25 mg/L). Interestingly, N-benzyl and N-phenyl substitution of S-(3,4-dichlorobenzyl)isothiourea resulted in a significant increased activity of the new derivatives to all tested Gram-positive bacteria (MICs ≥12.5 mg/L). Unlikely, these derivatives decrease the activity against only one of tested rods - *P. aeruginosa* (MIC 400 mg/L). On the other hand, what is the most important, the reduction of the MBC values of tested N-benzyl and N-phenyl substituted derivatives for all Gram-positive cocci as well as for *A. baumannii* and *S. maltophilia*

was observed. The highest activity against fungi and bacteria has been shown for S-(3,4-dichlorobenzyl)-N-benzyl-N'-methylisothiourea. The results also showed that the parent compound and its 8 derivatives were removed by efflux pumps present in *P. aeruginosa, E. coli, K. pneumoniae* and *E. cloacae* strains.
Conclusions:
Presented data indicated the structure-activity relationships among this class of agents. Obtained results justified the need to search for active substances to microorganisms among new S-(3,4-dichlorobenzyl)isothiourea derivatives.

The study was supported by the Foundation for the Development of Diagnostics and Therapy, Warsaw, Poland.

ANTIMICROBIAL EFFECTS OF A PROPRIETARY STANDARDISED GARLIC EXTRACT IQP-AS-105: A BROAD-SPECTRUM ACTIVITY ON GRAM-NEGATIVE, GRAM-POSITIVE AND FUNGAL MICROORGANISMS

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Abstracts
The effects of a proprietary garlic extract (IQP-AS-105), standardised against multiple organosulfur compounds such as allicin, ajoene and diallyl sulfides against 15 strains of Gram-negative, Gram-positive and fungal microorganisms were investigated by microdilution broth method. The susceptibility of drug-resistant microorganisms such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis*, multidrug-resistant *Klebsiella pneumoniae* and multidrug-resistant *Acinetobacter baumannii* were also studied. Results showed that the MIC of Gram-negative, Gram-positive and fungal strains ranged from 0.49-15.60 mg/ml, 1.95-15.60 mg/ml and 0.24-1.95 mg/ml respectively; MBC/MFC ranged from 0.98-15.60 mg/ml, 15.60-31.25 mg/ml, and 0.24-3.9 mg/ml respectively. IQP-AS-105 inhibited the growth of all the strains including drug-resistant strains. Bactericidal or fungicidal effects were found in 11 strains except for *Salmonella typhimurium, Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant *Enterococcus faecalis*. Results of this study indicate the potential use of IQP-AS-105 as a broad-spectrum antimicrobial agent that is effective against drug-resistant microorganisms. IQP-AS-105 showed relatively strong antimicrobial activities compared to other garlic extracts investigated in previous studies. Further research on the antimicrobial properties including interaction and synergism with antimicrobial drugs appears warranted.

INHIBITORY EFFECTS OF GARLIC METABOLITES ON CLOSTRIDIUM DIFFICILE

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The aim of this study was to examine the effects on vegetative growth and morphology as well as endospore formation and germination of Clostridium difficile under strictly anaerobic conditions in response to treatment with proprietary garlic extracts that are standardised against Ajoene (liquid) or Allicin (powdered) content.

Analyses were performed using automated optical density measurements under an anaerobic atmosphere to determine growth inhibition and subsequent scanning electron microscopy (SEM) imaging to qualitatively assess indications of cellular stress, biofilm and endospore formation. Escherichia coli (K12) was used for comparative studies as this strain has been used in studies into the inhibitory effects of garlic-derived metabolites.

Liquid garlic extract (10 µg/ml) containing 17.5% ajoene has the effect of doubling the lag phase of growth of C.difficile. The MIC of the same extract after 24h for C.difficile was 15 µg/ml. However, C.difficile was able to resume vegetative growth upon inoculation on fresh brain heart infusion agar even after incubation with 10 mg/ml of this extract. This led to the investigation of inhibition of endospore formation and germination in C.difficile by garlic extracts after treatment with sodium taurocholate. Endospore germination was inhibited by 5% at lower concentrations (25-75 μg/ml) of liquid garlic extract measured after 24h and by 20% using powdered garlic extract. The capacity of C.difficile to withstand elevated concentrations of garlic constituents is consistent with observations of durable endospores in this organism that can persist in hostile environments for up to 2 years.

SEM indicated that in the extract-treated groups there was a significant reduction in planktonic cells compared to the control and the development of biofilms was noted in both C.difficile and E.coli cultures.

The next phase of this research is to identify the various components in the extracts that exhibit antibacterial effects in order to enhance efficacy of inhibition. This will then be followed by studies in gut models to determine the impact of the gut environment on the activities of the extracts.

THE EFFECT OF SILVER NANOPARTICLES ON PSEUDOMONAS AERUGINOSA VIRULENCE FACTORS

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Introduction and Aim. Due to increase of the number of bacterial species resistant to multiple antibiotics, new alternative antimicrobial compounds, such as metal nanoparticles, are within the scope of many researches. Antibacterial potential of silver nanoparticles (AgNPs) has already been documented, however the mechanism of their action still requires clarification. Pseudomonas aeruginosa, an opportunistic human pathogen, is a frequent cause of infections in hospitalised patients, immunocompromised hosts and patients with cystic fibrosis. The Gram-negative P. aeruginosa is ubiquitous and well adapted for growth in an aquatic environment. It synthesizes an adhesive alginate extracellular matrix and forms biofilms very difficult in eradication. Our study shows the effect of AgNPs on virulence factors of this human pathogen.
Materials and methods. P. aeruginosa PAO1 was used in the study. Minimal inhibitory concentrations of silver nanoparticles that inhibit biofilm formation (MBIC) was estimated by standard two-fold dilution method in 96-well microtitre plates. Swimming motility of P. aeruginosa was examined on 0,3% agar plates and swarming motility on 0,6% agar plates supplemented with 0,05; 0,1; 0,25 or 0,5 x MBIC AgNPs. Twitching motility in presence of subinhibitory concentrations of AgNPs across the glass surface was analysed after staining with crystal violet. The change in biosurfactants production in presence of subinhibitory concentrations of nanosilver was estimated in cetyltrimethylammonium bromide (CTAB) agar test [1]. Effect of AgNPs on proteolytic activity of P. aeruginosa was examined after 24 h incubation with subinhibitory concentrations of nanosilver using azocasein as a substrate [2]. All the tests were conducted at least three times independently. Statistical analysis of the results in comparison to control sample without AgNPs was performed.
Results. MBIC of silver nanoparticles for P. aeruginosa was equal to 4 μg/ml. The change in bacterial motility in presence of AgNPs was also observed. There was significant decrease from 22% to 40% in swimming and from 22% to 29% in swarming motility in samples containing 0,1 – 0,5 x MBIC of AgNPs in comparison to P. aeruginosa PAO1 incubated without nanoparticles. No influence of AgNPs on twitching motility was observed. Addition of AgNPs did not cause significant change in amount of produced ramnolipids. Proteolytic activity of PAO1 was not significantly influenced by nanosilver.
Conclusion. Silver nanoparticles affect several virulence factors of P. aeruginosa associated with biofilm formation such as swarming and swimming motility. However no change in production of biosurfactants and proteolytic activity was observed.

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STAPHYLOCOCCUS AUREUS PROTEINACEOUS EXOPRODUCT ACTIVE AGAINST STAPHYLOCOCCUS EPIDERMIDIS BIOFILM

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Staphylococci are recognized as the most frequent causes of biofilm-associated infections, thanks to their ability to adhere on both eukaryotic cells and abiotic surfaces via cell wall. Staphylococcal biofilms display extraordinary resistance to antimicrobial killing and limit the efficacy of antibiotic therapy; thus surgical intervention is required to remove medical devices. Staphylococcus aureus and Staphylococcus epidermidis are prevalent species on skin and mucosae of animals and humans.
The interest in the development of alternative anti-infective approaches for the prevention and treatment of infections increased in recent years. Our rationale is to look for new antimicrobials inhibiting virulence, in particular bacterial adhesion and biofilm formation, rather than bacterial growth. This latter should exert a weaker selective pressure for the development of drug resistance.
In this work we examined the anti-biofilm activity of cell-free supernatants deriving from planktonic and sessile growths of S. aureus and S. epidermidis. Their effect on staphylococcal biofilm formation was evaluated both in static and dynamic condition. The dynamic condition was assessed in BioFlux System that precisely controls the flow of growth medium between two interconnected wells of a microtiter plate, in order to acquire sequential bright-field images of any growing biofilm. Physico-chemical characterization of active compounds was also performed. Preliminary size-exclusion chromatography was used to purify the active compound(s).
Data obtained have shown the anti-biofilm activity of the supernatants of S. aureus able to inhibit the biofilm formation, and to disrupt mature biofilms of S. epidermidis without affecting the bacterial viability. The anti-biofilm activity was both present in supernatants obtaining in planktonic and sessile growth conditions. Treatment with protease eliminated anti-biofilm activity in supernatant. No surfactant activity was detected. Protein profiles of fractions derived from chromatography were analyzed by SDS-PAGE and tested for their anti-biofilm activity.
Some active fractions derived from chromatography with a discrete number of proteins having anti-biofilm activity were identified. The active compound is probably an exoprotein with molecular weight higher than 50,000 Da. The isolation of active molecule necessarily requires additional steps of purification.
In conclusion, in order to understand the mode of action of active molecule, its identification is a fundamental prerequisite.

EFFICIENCY OF PHAGE COCKTAILS IN THE INACTIVATION OF SALMONELLA TYPHIMURIUM IN BIVALVE MOLLUSCS

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Abstract
Salmonella enterica subsp enterica serovar Typhimurium is the most frequent causative agent of human gastroenteritis, after consumption of contaminated seafood. Bacteriophages are safe bio-controlling agents, and have been recognized in aquaculture for their pathogen reduction capabilities. The aim of this study was to evaluate the efficiency of cocktails of two and three phages of S. Typhimurium (SE-1, SE-2 and SE-5) to control Salmonella in bivalve molluscs. All phages were effective against S. Typhimurium, being potential candidates, used individually or in cocktails, as agents for the biological control of infections transmitted to humans by consumption of bivalve molluscs. The maximum of bacterium inactivation with SE-1 phage was 1.8 log achieved after 4 h of phage therapy. After 12 h of treatment, the rate of inactivation was still considerably high (1.3 log). With SE-2 and SE-5 phages, the maximum of bacterium inactivation was 2.4 and 2.5 log, respectively, after 12 h of phage therapy. The rate of inactivation with SE-2 and SE-5 after 4h of treatment was similar SE-1 phage. The cocktail SE-

2/SE-5 was more effective (reductions of 2 log after 4 h and 2.7 log after 12 h) than the SE-2 and SE-5 phages used alone. However, the efficiency of SE-1/SE-2, and SE-1/SE-2/SE-5 cocktails was similar, respectively, to SE-2 and SE-5 phages alone. The efficiency of SE-5 phage alone was higher than the phage cocktail SE-1/SE-5. All phages remained viable for a long time in marine water. The SE-5 phage presented a larger burst size and a shorter latent period (188 and 15 min, respectively) than the other two phages (33 and 20 min and 39 and 20 min, respectively, for SE-1 and SE-2 phages). The three phages revealed to be efficient in the inactivation of S. Typhimurium being potential candidates, used individually or in cocktails, as agents for the biological control of infections transmitted to humans by consumption of bivalve molluscs, and the use of Salmonella phages with high burst sizes and short lytic cycles increase the efficiency of phage therapy.

PHAGE COCKTAILS AS AN ALTERNATIVE TO INACTIVATION OF ENTEROBACTER CLOACAE

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Abstract
Enterobacter cloacae is part of the normal flora of the gastrointestinal tract of 40-80% of people. This opportunistic microorganism is capable of causing infection in debilitated and hospitalized patients. E. cloacae is resistant to a broad number of antibiotics therefore infections caused by this bacterium are difficult to control. Phage therapy may be a useful tool to control infections caused by resistant bacteria. Three previously isolated phages E-2, E-3 and E-4 produced on Enterobacter cloacae were used to examine survival and host-phage dynamics. The survival was determined in PBS through quantification by soft agar overlay technique. The host-phage dynamics was characterized in tryptic soy broth, through quantification of phages by soft agar overlay technique and host quantification in TSA medium. The concentration of E-2 decreased by two orders of magnitude in the first 105 days. The concentration of E-3 decreased by one order of magnitude in the first 20 days and reached a plateau until 77 days. Afterwards, the phage titer decreased by three orders of magnitude until 156 days. E-4 concentration only decreased by one order of magnitude after 255 days. The results show that the growth of the E. cloacae was inhibited by the three phages, resulting in a decrease of ≈ 3 log after 4-10 h of incubation. The use of cocktails with two or three phages was significantly more effective, with reductions of ≈ 4 log after 2 hours. Phages E-2, E-3 and E-4 showed an efficient inactivation of E. cloacae, being potential candidates as agents for the control of nosocomial infections caused by Enterobacter cloacae.

SecA, AN ATTRACTIVE ANTIBACTERIAL TARGET

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The rapid rise of antibiotic-resistant bacteria is one of the major concerns in modern medicine. Therefore, there is an urgent need for new antibacterials to treat bacterial infections, which are preferably directed against alternative bacterial targets. One such potential target is the preprotein translocation motor SecA. SecA is a peripheral membrane ATPase and a key component of the Sec-dependent protein translocation pathway, which is the major route for bacterial protein export across or into the cytoplasmic membrane. Since SecA is essential for bacterial viability, ubiquitous and highly conserved in bacteria, but not present in eukaryotic cells, it represents an attractive antibacterial target. Using an *in silico* approach, several potentially druggable and conserved pockets on the surface of SecA were defined. We show that three of these potentially druggable sites are important for the *in vivo* function of SecA. Via structure-based virtual ligand screening, ~500,000 commercially available small molecules of the ChemBridge compound library were virtually screened against a predicted druggable pocket in the preprotein binding domain of *E. coli* SecA. The 1040 top-scoring molecules were tested *in vitro* for inhibition of the translocation ATPase activity of *E. coli* SecA. Four inhibitors of the translocation ATPase, and not of basal or membrane ATPase, were identified with IC_{50} values <65 μM. The most potent inhibitor showed an IC_{50} of 24 μM. Two compounds were found to possess weak antibacterial activity against *E. coli* (IC_{50} ~200 µM), while two other compounds showed moderate antibacterial activity against *S. aureus* (IC_{50} ~90 µM).

NOVEL SUICIDE SUBSTRATES FOR IMPROVED PATHOGENIC BACTERIAL IDENTIFICATION IN CLINICAL SAMPLES

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INTRODUCTION

The continued emergence of resistant bacteria has increased the need for new, rapid, economical and user-friendly bacterial detection and identification methods. Their development is especially significant for clinical applications to enable the selection of the most effective antibiotic to control specific pathogenic bacteria. Apart from its clinical use, bacterial detection is also widely used in food quality control and in environmental monitoring, in order to ensure that food and water are bacteria-free. There are many bacterial detection methods available, covering both conventional and sophisticated instrumental methods, such as microscopic, culture, biochemical, immunological and genetic; however, most of these methods are time-consuming, costly, and require fully trained or experienced personnel for their operation.

The use of synthetic enzymatic substrates continues to offer huge potential for accessible bacterial detection and identification, because it is more rapid, economical, user-friendly (no training required), and is suitable for routine analysis. This method works by incorporating bacterial-specific enzymatic substrates, attached to a chromogen or fluorogen, into the culture medium [1]. The chromogenic or fluorogenic response will be exhibited in the presence of specific strains of bacteria. However, in this method, a similar enzyme may be secreted by different strains of bacteria and can result in false positive results. An appropriately selective suicide substrate incorporated into the culture medium can prevent the growth of interfering bacteria. For instance, when investigating faecal samples to identify the bacteria responsible for severe food-poisoning, alafosfalin (a known suicide substrate) allows the growth of *Salmonella* and other pathogenic species, while preventing the growth of camouflaging commensal bacteria such as *E. coli* [2].

The aim of this work was to develop inhibitory suicide substrates for specific organisms to facilitate the growth of other bacterial species of interest.

MATERIALS AND METHODS

Peptide coupling reagents, isobutyl chloroformate and *N*-methylmorpholine in dry solvents (DCM and THF), and diisopropylethylamine (for neutralisation) were used. Synthesis of peptide-mimic compounds (fosfalin and/or ⍰-chloro-alanine containing derivatives) using an established peptide coupling method were performed at University of Sunderland.

Bacterial evaluation of three peptidomimic compounds was performed at Freeman Hospital, Newcastle upon Tyne, against 20 strains of National Collection of Type Culture (NCTC) bacteria.

RESULTS AND DISCUSSION

Selective and potent inhibition of bacterial species, such as *Staphylococci*, *E. coli*, *Enterococci*, *Yersinia* and *Klebsiella,* was observed at concentrations of less than 1.0 mg/L. The minimum inhibitory concentration (MIC) of the tested peptidomimetic compounds against 20 NCTC strains is shown in Table 1.

Table 1: The minimum inhibitory concentration (MIC) of the tested peptidomimetic compounds, with significantly lower MIC values against certain of the 20 NCTC strains, will be presented in a table.

CONCLUSIONS

Selective and potent inhibition of a range of commensal bacteria was demonstrated by the peptidomimetic compounds. These compounds offer potential as suicide substrates for selective bacterial inhibition. The inhibition of commensal species responsible for overgrowth and masking of infecting bacteria present in low numbers would facilitate the identification of pathogenic species, improve accurate clinical diagnoses and support antimicrobial stewardship programmes.

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COMPARISON OF THE ANTIBACTERIAL EFFECT OF SILVER AND ZINC OXIDE IN SOLUTIONS AND ON COATED SURFACES

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Aims: The aim of this study is to assess the antibacterial effect of Silver (Ag) and Zinc (ZnO) nanoparticles in solution, and on coated surfaces against *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms.

Methods and Results: A CDC biofilm reactor was used to form biofilms of *S. aureus* and *P. aeruginosa* on polycarbonate coupons for 48 hours using a continuous flow of nutrient broth. A chequerboard method was then used to treat biofilms with solutions containing combinations of Ag and ZnO ranging from 8 to 100 µg/ml for 24 hours. The biofilms were then enumerated and the Fractional Inhibitory Concentration (FIC) established. Solutions containing 12 µg/ml of Ag and 8 µg/ml of ZnO for *S. aureus* and 32 µg/ml of Ag and 25 µg/ml of ZnO against *P. aeruginosa* were found to be most effective, having an additive effect.
Reductions in biofilms of *S. aureus, Methicillin Resistant Staphylococcus aureus* (MRSA) and *P. aeruginosa* (type and clinical strains) were then established. Biofilms were reduced by 3.91 $\log_{(10)}$ cfu/ml, 3.81 $\log_{(10)}$ cfu/ml, 3.66 $\log_{(10)}$ cfu/ml and 3.68 $\log_{(10)}$ for *S. aureus*, MRSA, *P. aeruginosa* type and clinical isolates respectively.
Silicon wafers coated with a thin film of Ag and ZnO of ~100nm were placed in the CDC biofilm reactor. Reductions of 0.44 $\log_{(10)}$ cfu/ml for Ag and 0.41 $\log_{(10)}$ cfu/ml for ZnO were observed against *P. aeruginosa*. For *S. aureus*, reductions of 0.88 $\log_{(10)}$ cfu/ml for Ag and 0.73 $\log_{(10)}$ cfu/ml for ZnO were obtained.
Conclusion: Treating a biofilm after formation with a nanoparticles solution containing 12/8 µg/ml or 25/32 µg/ml of Ag and ZnO respectively is more effective than preventing growth of a biofilm on a surface when a thin film coating has been applied. This may be due to the Zn and Ag ions on a coated surface being released at a slower rate in the media over 24 hours. However, when a biofilm is treated with a solution containing nanoparticles, the biofilm is exposed to an instant maximum dose, exerting a greater antimicrobial effect.
Significance of study: With antibiotic resistance remaining a problem, it is vital that novel alternative treatments are established. The use of nanometals, in combination and via different delivery systems, such as coated surfaces and disinfectants to treat biofilms may be a solution in preventing the transmission of Hospital Acquired Infections.

ANTI-INFECTIVE MOLECULES FROM MARINE BACTERIA AGAINST THE MOST COMMON CYSTIC FIBROSIS PATHOGENS: *S. AUREUS* AND *P. AERUGINOSA*

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Cystic fibrosis (CF) is a hereditary disease that affects the normal function of epithelial cells, especially in the lungs and digestive system, causing progressive disability. Recurrent and chronic respiratory tract infections in CF patients represent the primary cause of morbidity and mortality. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most common bacterial pathogens isolated from the CF respiratory tract where they cause persistent infections. CF infections treatment is hampered by the ability of bacteria to form biofilms intrinsically resistant to common antibiotics used in therapy. Impeding biofilm formation is an important step towards infection control and they might be considered major target for the development of novel therapeutic agents. Many bacteria secrete anti-biofilm molecules that function regulating biofilm architecture or mediating the release of cells from it during the dispersal stage of life cycle.
Cold-adapted marine bacteria represent an untapped reservoir of biodiversity able to synthesize a broad range of bioactive compounds, including anti-biofilm molecules.
The anti-biofilm activity of 20 cell-free supernatants derived from sessile and planktonic cultures of Antarctic bacteria belonging to *Pseudoalteromonas* and *Psychrobacter* species were tested against *S. aureus* and *P. aeruginosa* strains. A preliminary physico-chemical characterization was also performed.
Reported results demonstrate that we have selected supernatants, from cold-adapted marine bacteria, containing non-biocidal agents able to destabilize *S. aureus* and *P. aeruginosa* biofilm matrix without killing cells. In particular, Antarctic supernatants are active to disaggregate the biofilm produced by all *P. aeruginosa* and by almost all *S. aureus* tested strains. In such cases, the production of anti-biofilm activity is related to the growth condition (sessile or planktonic). The physico-chemical characterization of the supernatants highlighted the presence of molecules of different nature that act inhibiting biofilm formation (proteins, surfactants or polysaccharides).
The present study reports about the marine bacterial culture supernatants that inhibit the biofilm formation of *S. aureus* and *P. aeruginosa*. Some of them are also able to impair the initial attachment of the bacterial cells to the surface, acting as surfactant molecules. Initial attachment to a surface is an important function in biofilm formation because it creates a favorable condition to bacteria for further establishment. Most promising supernatants will be further analysed in order to characterize the active components. Thus they could be combined with a conventional antibiotic to eradicate biofilm infection.

SYNERGISTIC EFFECT OF TEA TREE OIL AND AMPHOTERICIN B AGAINST *CANDIDA ALBICANS*: AN ALTERNATIVE TREATMENT.

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Candidosis is the most important cause of fungal infections in humans. The yeast *Candida albicans* can form biofilms, and it is known that microbial biofilms play an important role in human diseases being very difficult to treat. The prolonged treatment with drugs has often resulted in failure and resistance. Due to the emergence of multidrug resistance, alternatives to conventional antimicrobial therapy are needed. The synergistic or additive effect produced by the combination between essential oils and antimicrobial drugs was referred as a strategy for counteracting microbial development (Wagner and Ulrich-Merzenich, 2009). In this work a possible synergistic anti-*Candida* effect of of *Melaleuca alternifolia* (tea tree oil, TTO) oils of two different batches (TTO_1 and TTO_2) and amphotericin B towards *Candida albicans* has been studied with the aim of finding minimum effective dose that maintain antifungal activity but minimize amphotericin side effects and down the onset of microbial resistance.

First the minimum inhibitory concentrations (MIC) of the individual oils was determined by using the microdilution method following CLSI guidelines. The checkerboard assay was carried out as described by Lorian (2005) with slight modifications in order to evaluate the synergy between TTOs and amphotericin B (AppliChem GmbH, Germany) against *C. albicans* ATCC 48274: every possible combinations of seven serial two fold dilution of TTO (from 0,06% v/v to 4% v/v) and antibiotic (from 0,125μg/ml to 4 μg/ml) were tested. All experiments were conducted in triplicate. The components of the TTO oils have been previously characterized by chromatographic analyses.
The results obtained showed the occurrence of pronounced fungicidal synergism [FFC (fractional fungicidal concentration) = 0.32 for TTO_1 and FFC= 0.28 for TTO_2] in which a low concentration of TTO (0.06% v/v) enhances the action of amphotericin B at a lower dose (0.125 μg/ml) compared to amphotericin B used alone (1μg/ml) against *C. albicans*. The *in vitro* data of the present study suggest the possibility to reduce the effective dose of antibiotic for treatment in the clinical setting decreasing the adverse effects of antibiotics. Further studies are needed to evaluate the toxicology of single components and their combination and to investigate the efficacy of this combination in animal models.

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SYNERGISTIC EFFECT OF RHAMNOLIPIDS AND CHLORHEXIDINE OR AMPHOTERICIN B AGAINST BIOFILM POPULATIONS

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Pathogenic microorganisms growing in a biofilm have usually significantly higher resistance to antimicrobial agents. Published data indicate an increase in resistance by up to 3 orders of magnitude. One of the possibilities overcoming these problems is to find such additive substances that can augment the activity of antimicrobial compounds without induce development of resistance. Particular attention is paid to compounds that are able to eradicate biofilms of different pathogenic microorganisms.
In this context, we focused on the preparation of microbial rhamnolipids, produced by *Pseudomonas aeruginosa*, *Enterobacter asburiae* and *Acinetobacter calcoaceticus*. Rhamnolipids are surface active compounds with proven ability to inhibit biofilm formation or disrupt mature biofilm. We studied these substances as potential modulators of antibacterial activity of chlorhexidine against Gram-positive bacterium *Rhodococcus erythropolis* and antifungal activity of amphotericin B against opportunistic pathogenic yeasts *Candida parapsilosis* and *Trichosporon cutaneum.* Cultivations of suspension microbial populations were performed in the shaken Erlenmeyer flasks. Experiments with biofilm populations were carried out in polystyrene 96-well microculture plates. Cellavista (SynenTec Bio Services GmBH), an automated light / fluorescence microscope which allows capturing images of biofilms directly in microculture plates and their subsequent processing by image analysis, was used for the study of biofilms.
Minimum inhibitory concentrations (MICs) of the tested compounds (chlorhexidine, amphotericin B and rhamnolipids) were determined. As expected, there were considerable differences in the sensitivity of microorganisms growing in suspension or as biofilm populations. For example, when the MICs of amphotericin B for suspension populations of *C. parapsilosis* and *T. cutaneum* ranged in units of mg / L and for rhamnolipids ranged in tens of mg / L, in the case of biofilms values ranged in the hundreds to thousands of mg / L. Fractional inhibitory concentrations (FICs) for mixtures: chlorhexidine vs. rhamnolipid and amphotericin B vs. rhamnolipid were determined simultaneously with MICs. FICs correspond to the concentrations of each compound in the mixture whose effect is the same as effect of single compound used at MIC. FIC indexes (FIC_i) which were used to characterize the type of interaction between the substances were calculated based on FICs values determined above. It was demonstrated that the application of antimicrobial substances together with rhamnolipids creates a synergistic effect on the inhibition of microbial growth. The same type of synergies was demonstrated also in the case of biofilm populations. A suitable ratio of chlorhexidine and rhamnolipid (produced by *P.*

aeruginosa) allows up to 80 % reduction in coverage of polystyrene plates by *R. erythropolis* in comparison with the chlorhexidine applications only. Simultaneous application of rhamnolipid and amphotericin B significantly reduced cell adhesion even at lower doses of antibiotics. Rhamnolipid in the amount of 50 % of MIC and amphotericin B in the amount of 25 % of MIC caused 75 % decrease of adhesion of *T. cutaneum.* The same ratio of both substances allows an adhesion of *C. parapsilosis* comparable to the application of amphotericine B at MIC. FIC of amphotericin B and rhamnolipid found for biofilm *T. cutaneum* and *C. parapsilosis* caused efficient eradication of mature biofilm.

INTRAMAMMARY INFUSION OF A LIVE CULTUREOF LACTOCOCCUS LACTIS IN LACTATING EWES WITH MASTITIS BY COAGULASE NEGATIVE STAPHYLOCOCCI: A PRELIMANARY FIELD STUDY

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Mastitis is an inflammation of the mammary gland that causes a reduction in the quality/quantity of milk output, increased veterinary expenses, and, due to the antibiotic therapy, increased risk of residues in the milk/meat and of drug resistance and, consequently the possibility of damage to public health. Development of alternative antimicrobial agents is encouraged in an age of mounting antibiotic resistance. In recent years interest has increased for bacteriocins and lactic acid bacteria as new antibiotic alternatives.In our study we have assessed the efficacy of a live culture of anisin-producer *Lactococcus lactis* in the treatment of mastitis causes by coagulase negative staphylococci (CNS) in lactating ewes. We have also assessed the oxidative stress by measuring the free radical production in blood.

Twenty ewes were enrolled and the health status of the glands were assessed. Both bacteriological and somatic cell count (SCC) results were considered: six ewes presented no intramammary infection (IMI), seven had unilateral IMI and seven had bilateral IMI. Twenty-one out of 40 udder halves were culture positive. The major bacterial pathogen was *Stapylococcus epidermidis*. The infected ewes were randomly divided into three groups and the udder halves with bacterial infection underwent to different treatments: in groups 1 and 2, the udder halves (N°=5 and N°=13, respectively) were daily intramammary infused with sterile saline solution (group 1) or with a live culture of *L. lactis* subsp. *lactis* (group 2) for a total of three consecutive days; in group 3, the udder halves (N°=3) were untreated. Foremilk samples were taken before any treatment was started (T0) and at days 1, 2, 3, 7, 14, 30 and 45 after the first intramammary infusion (T1, T2, T3, T7, T14, T30 and T45, respectively). Milk samples were analysed microbiologically and for SCC. Blood samples were also collected from all groups at T0, T7 and T14, and the free radical levels were determined by d-ROMs test (Diacron, Italy).

We observed that the third dose of *L. Lactis* (group 2) was able to make a temporary reduction of the pathogen growth. Milk samples were collected from group 2 at T3 and 92% udder helves had negative microbiological results, although the SCC was high (>2000 000 cells/ml) in all samples. In turn, 20% and 67% udder helves from group 1 and 3 were culture negative at T3, respectively. Progressively, the bacterial pathogens recolonized the gland in all treated groups. No culture negative samples were collected from group 1 and only one udder half was microbiologically negative, although the SCC was high (> 2 000 000 cells/ml), at T45. In the untreated group (group 3), 67% udder helves were still culture negative at T45; however, the SCC was high in all milk samples. Blood analysis showed no oxidative stress in any group.

In our preliminary field study and under the above experimental conditions, the benefits of the live culture of *L. lactis* were temporary and disappeared within few days after the last dose.The study was financial supported by the Italian Ministry of Health, grant number RF-2010-2373040.

DICTYOSTELIDS WITH BROAD ANTIBIOFILM ACTIVITIES

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Bacteria in nature commonly form complex biofilm communities consisting of cells encased in an extracellular matrix. Biofilms pose significant problems for medicine, agriculture, and other industries because conventional antimicrobials and disinfectants have little to no effect against them. However, organisms that feed on bacteria may have tools to disrupt biofilms in order to access their prey. Dictyostelids, also known as cellular slime molds, are benign myxamoebae that feed on bacteria in soil bio-webs and on other surfaces. In this work, we tested the potential of isolates representing 6 dictyostelid species to destroy biofilms formed on glass coverslips and polycarbonate filters. The abilities of dictyostelid spores to germinate on biofilms and the emerging vegetative cells to grow, divide, and differentiate were monitored microscopically for two hallmarks of activity: 1) predation on bacteria by dictyostelid cells and 2) characteristic stages of multicellular development (streaming and fructification). We found that all dictyostelid isolates tested could survive when presented biofilm-embedded bacteria as a food source. Not surprisingly, however, our survey data suggested different pairings of species (predator/prey) might exhibit variations in the efficiencies of biofilm destruction and dictyostelid development. To investigate further, we conducted quantitative experiments using biofilms of *Klebsiella Staphylococcus, Pseudomonas and Erwinia*, pathogenic bacteria that present challenges to medicine and agriculture. The quantitative studies confirm the ability of myxamoebae to destroy biofilms and are consistent with the hypothesis that isolates may exhibit variability in the mechanisms they employ to access biofilm-enmeshed bacteria. We will discuss how these newly discovered properties of dictyostelids might be useful in the management of bacterial biofilms.

CONTROL OF *VIBRIO PARAHAEMOLYTICUS* CONTAMINATION IN THE BLUE MUSSEL (*MYTILIS EDULIS*) BY LOW NUMBERS OF VIBRIOPHAGES.

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Vibrio parahaemolyticus is a Gram-negative bacterium which naturally occurs in marine and estuarine environments and is frequently isolated from seafoods including the blue mussel (Mytilis edulis). The organism is an important human pathogen which can cause gastroenteritis when consumed in raw or partially-cooked seafood [1]. The antimicrobial susceptibility of *V. parahaemolyticus* has changed over the past decade and more extensive resistance has emerged amongst both clinical [2] and environmental isolates [3]. Due to the increasing challenge of antibiotic resistance in *V. parahaemolyticus*, the need for alternative control mechanisms such as the use of bacteriophage has been prompted. This study aims to determine the efficacy of phage-mediated control in reducing or eradicating *V. parahaemolyticus* when tested in the laboratory or *in vivo*. The experimental effects of bacteriophages isolated from enriched seawater samples collected from the river Humber against *V. parahaemolyticus* was determined in Tryptone Soya Broth (+ 3% NaCl) incubated for 24 hours. In addition, a microcosm experiment with live blue mussels in 2 litres of seawater using similar methodology was established. The culture population of *V. parahaemolyticus* was reduced by an average of 1.2 log after 24 hours of incubations at 30°C *in vitro*, whilst in mussel flesh the population of *V. parahaemolyticus* was reduced by an average of 1.4 log and 5.7 log, 72 hours after the addition of 10 ml and 20ml of bacteriophage cocktail containing 12 different phages respectively. Our *in vitro*/*in vivo* study of bacteriophage in controlling *V. parahaemolyticus* indicates that a cocktail of specific phages targeted against strains of *V. parahaemolyticus* possess valuable potential in their use as decontamination agents in mussel post-harvest processing.

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COLD PLASMA – ALTERNATIVE TECHNOLOGY TO ANTIBIOTICS?

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Low temperature atmospheric plasma (cold plasma) represents an exciting new research direction. Cold plasma when compared with hot plasma has the advantage that they operate under 40°C and therefore allow *in vivo* applications without damaging the surrounding tissue. Low temperature plasma – which generates a physical and chemical mixture of electrons, ions, agitated atoms and molecules, radicals, reactive species and UV radiation – has antibacterial, antimycotic and antiviral properties.
In a project led by the Max Planck Institute in Germany, Adtec's MicroPlaSter cold plasma technology has been used in clinical trials with the followng results published;

1. Anti-microbial – kills the bacteria in infected wounds/infections/ulcers leading to shorter healing time (reduced hospital costs)
2. Reduces pain – relief for the patients/ reduces need for pain medicine.
3. Safe treatment with no side effects reported – MicroPlaSter was used to treat over 300 patients over a 7 year period.

4. Physical action – microorganisms less likely to develop resistance. Can be considered as an alternative technology to antibiotics.

ASSESSMENT OF THE ACTIVITY OF A LIGHT-ACTIVATED ANTIMICROBIAL SURFACE

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Nosocomial infections are a major problem in modern healthcare because they cause fatalities and increase expenditure. There is evidence that strongly suggests that environmental surfaces are a major factor in transmission of nosocomial pathogens. Deploying antimicrobial surfaces in hospital wards could reduce the role environmental surfaces play as reservoirs for pathogens. The aim of this study was to assess the antimicrobial activity of a light-activated surface. The surface was a medical grade silicone incorporating crystal violet, methylene blue, and 2 nm gold nanoparticles. A viability assay was performed to assess the microbicidal activity of the surface against unicellular microorganisms. A significant reduction in viable counts was seen in *Staphylococcus epidermidis*, *Saccharomyces cerevisiae*, and MS2 Bacteriophage after treatment on the surface in the presence of white light. A migration assay demonstrated that, in the presence of white light, the surface significantly inhibits growth of the fungus-like organism *Pythium ultimum* and the fungus *Botrytis cinerea*, but was ineffectual against *Rhizoctonia solani*. Microscopy studies were performed to gain an insight into the mechanisms of action of the surface. Atomic Force Microscopy (AFM) demonstrated that exposure to the surface under white light causes significant alterations to the surface of *Staphylococcus epidermidis*, which is indicative of cell envelope damage, and also demonstrated that contact with the surface can cause *Staphylococcus epidermidis* to aggregate into masses. Transmission Electron Microscopy (TEM) confirmed the AFM findings and also demonstrated that discrete surface linkages connect these aggregated cells to each other. Therefore, the study has demonstrated that the surface has a broad antimicrobial activity under white light, and that the surface treatment causes bacterial envelope damage and cell aggregation.

Day 2: Anti-Microbial Peptide Strategies

Invited Speakers Abstracts

From Host-Defense Peptides To New Antibiotics

Dr Paul R. Hansen, University of Copenhagen, Denmark

Antimicrobial peptides hold promise as the next generation of antimicrobial agents. However, the potential is weakened by their susceptibility to proteolytic degradation, poor oral activity, toxicity and high cost. Our research interest is in determining the structure/activity relationships of these antimicrobial peptides, which enables us to better understand their mechanism of action and facilitates the development of lead antimicrobial agents. As an example, in this talk I will focus on our work on anoplin and analogues, a decapeptide isolated from the Japanese wasp Anoplius samariensis. I will describe the elucidation of their structure/activity relationships, and our efforts towards developing them into antibiotics.

Antimicrobial peptides (AMPs) are found in all multicellular organisms and display activity against a broad spectrum of pathogens, including Gram-positive and Gram-negative bacteria. Antimicrobial peptides hold promise as the next generation of antimicrobial agents. However, the potential is weakened by their susceptibility to proteolytic degradation, poor oral activity, toxicity and high cost. Our research interest is in determining the structure/activity relationships of these antimicrobial peptides, which enables us to better understand their mechanism of action and facilitates the development of lead antimicrobial agents. As an example, in this talk I will focus on our work on anoplin and analogues, a small decapeptide isolated from the Japanese wasp *Anoplius samariensis* [1-3]. I will describe the elucidation of their structure/activity relationships, and our efforts towards developing them into antibiotics.

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1.K. Konno, M. et al. (2001) Anoplin, a novel antimicrobial peptide from the venom of the solitary wasp Anoplius samariensis. *Biochim Biophys Acta*, **1550**, 70-80.
2.Jens K. Munk, Christian Ritz, Frederikke P. Fliedner, Niels Frimodt-Møller, and Paul R. Hansen (2014) Novel method to identify the optimal antimicrobial peptide in a combination matrix, using anoplin as an example. *Antimicrobials Agents and Chemotherapy*, **58**, 1063-1070.
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Anti-microbial cationic peptides as a strategy for innovation in global health therapeutics for human infectious diseases

Dr Hussin Rothan, Researcher, Faculty of Medicine, University of Malaya, Kuala Lumpur , Malaysia

Most of the living organisms produce antimicrobial peptides (AMPs) to protect themselves against pathogens. Diverse structures AMPs have been identified with different activities against human viruses, bacteria, fungi, protozoa parasites and neoplastic cells. The molecular structures of AMPs are mostly alpha helical, beta-sheets and loops. Therefore, the AMPs are considered as promising antibiotic alternatives with important biotechnological applications. Our studies showed considerable inhibition of some AMPs against Flaviviruses, alphaviruses and pathogenic bacteria. The manufacturing obstacles were addressed in our studies by producing the AMPs in E. coli as inclusion bodies that were properly refolded to retrieve the antimicrobial activities.

Octapeptins revisited

Dr Tony Velkov, Monash Institute of Pharmaceutical Sciences, Victoria, Australia

The octapeptins are a family of naturally occurring cyclic lipopeptide antibiotics with a broad antimicrobial Spectrum against multidrug-resistant 'superbugs'. Not unlike the polymyxins, interest in their development as therapeutics waned as β-lactam antibiotics were favoured at the time due to their low toxicity and ideal pharmacological profiles. For the first time our novel approach will interface the chemistry and biology of these important antibiotic compounds to med-chem out liabilities such as nephrotoxicity whilst concomitantly improving the antimicrobial activity and spectrum, thereby creating the foundations for a new generation of safer and more efficacious antibiotics.

Molecular dynamics simulations reveal mechanistic details of polymyxin penetration into both membranes of E.coli

Dr Syma Khalid, Senior lecturer in Computational Chemistry, University of Southampton, Southampton, UK

We present, to our knowledge, the first molecular dynamics simulation study of an antimicrobial peptide, with both membranes of E.coli. Our simulations reveal the effects of Polymyxin B1 (PMB1) binding on the physical properties of each membrane. Thus they are able to identify potentially different mechanisms for membrane disruption by PMB1. Peptide aggregation and insertion of one peptide tail was observed in the outer membrane. In contrast, PMB1 peptides insert readily as monomers, accompanied by water penetration into the inner membrane. Our simulations demonstrate the importance of capturing relevant details of biological complexity, in molecular models of biological membrane systems.

Preclinical development of a novel synthetic antimicrobial peptide for lung infections and sepsis

Dr Alessandro Pini, Dept. of Medical Biotechnology, University of Siena, SetLance srl Toscana Life Sciences, Italy

The antimicrobial peptide M33 was selected by a synthetic library and optimized for stability and antimicrobial performance. It resulted active against multiresisitant Gram-negative bacteria including clinical isolates of P. aeruginosa, K. pneumonia, A. baumannii and many enterobacteriaceae. It protected animals from deadly infections in models of sepsis and pneumonia. It is currently under preclinical characterization for the development of a new drug against microbial infections and expected to enter the clinic in the next two years.

Oral Presentation Abstracts

IN OVO ADMINISTRATION OF DCATH2 PEPTIDE: MONITORING OF UPTAKE BY MICROPET/CT IMAGING AND EFFICACY IN A CHICKEN SALMONELLOSIS MODEL

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Mammalian host defense peptides (HDPs) exhibit a wide array of immunomodulatory functions on immune cells, including apoptosis inhibition, recruitment, wound healing, enhanced antigen uptake and presentation and may so boost innate immunity. This study was aimed to determine in ovo efficacy of chicken HDP analog DCATH-2 to reduce experimentally induced salmonellosis in young broiler chickens. First, we studied the uptake of ^{89}Zr-radiolabeled CATH2 peptide via the amnion of 18-day old embryos by positron emission and computed tomography (MicroPET/CT) and by ex vivo biodistribution analysis. These showed a significant uptake of peptide in stomach, lungs and intestine within 24 hours. Next, an efficacy study was performed by injecting DCATH-2 (1mg/kg bodyweight) into the amnion of 18 day-old Ross 308 embryos that were challenged subcutaneously at 4 days post hatch with 5×10^6 CFU of avian pathogenic *Salmonella enteritidis* PT13a. DCATH-2 treatment increased peripheral blood leukocyte and heterophil counts at 48h p.i. and greatly reduced mortality (50%), the number of birds with lesions (69%) and lesion severity (67%) at 7 days p.i. These findings show that in ovo administration of HDP-based anti-infectives are of potential use for veterinary medicine.

THE NON-ANTIBIOTIC CONTROL OF BIOFILMS ON ABERRANT WOUNDS

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Surface Sciences Research Centre and Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK and Scapa Healthcare, Manchester, UK

Wounds that demonstrate non-healing and minimal response to topical and systemic antimicrobials, which deteriorate further once the antimicrobials are removed, are 'infected' with a pathogenic biofilm. Pathogenic biofilms are responsible for enhancing chronic inflammation in a wound. Chronic inflammation leads to a delayed response to healing and an increased propensity to infection. The sessile microbes growing within a wound biofilm, when compared to their planktonic counterparts, are well known to be recalcitrant to the majority of commercially available antibiotics and antiseptics. Biofilms have inherent tolerance to antibiotics and antimicrobial agents in general. As antibiotics by themselves are reported to have limited response in biofilm infected chronic wounds it is being recommended that their usage be restricted to use for only a short duration of time. Consequently next generation antimicrobials are required for use in the control of wound biofilms. At present wound biofilms are being removed with the use of sharp debridement with a curette, or more formal means of surgical debridement. However, as biofilms regrow very quickly in the wound a combination approach utilising multiple antiseptic and anti-biofilm strategies should be employed to suppress regrowth. Combined anti-biofilm strategies include antiseptic wound cleansers, topical antiseptics, and appropriate wound dressings that work synergistically to suppress the wound biofilm. Furthermore a rotating course of selective antiseptics will be advantageous in biofilm prevention and reduction. The focus of this presentation is to provide strategies that could be employed to suppress biofilms in wounds until the next generation anti-biofilm technologies are available.

MFM 501: A NEW PYRROLIDINE DERIVATIVE AGAINST MULTIDRUG-RESISTANT MRSA ISOLATES

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Methicillin-resistant Staphylococcus aureus (MRSA) is a nosocomial-related, Gram-positive bacteria that have been known to display multidrug-resistance (MDR) properties towards a wide range of structurally-unrelated antibiotics and antimicrobial agents. Currently, only a handful of antibiotics could inhibit this dangerous pathogen. Hence, a dire need for a new and alternative source of antimicrobials is very crucial to prevent or at least reducing MRSA infections worldwide. Previously, codonopsinine, a polyhydroxy pyrrolidine alkaloid isolated from *Codonopsis clematidea* are known to display remarkable antibiotic and hypotensive activities while does not affect the central nervous system. The aerial parts of this plant has been used traditionally to treat liver disease, hepatitis, jaundice and are used in combination with other folk medicine to improve hepatic functions. In the present study, we have successfully synthesized 30 derivatives of codonopsinine which represent interesting electron rich functional group identified to be responsible for many pharmacological properties. One derivative, MFM 501, showed good inhibitory activity with MIC value of 15.6 to 31.3 µg/ml against 43 MDR MRSA isolates. In addition, MFM 501 showed low cytotoxicity activity towards three normal cell-lines (WRL-68, Vero and 3T3) with IC_{50} values at > 625 µg/ml. Selectivity Index (SI) of MFM 501 gave a value of > 10 suggesting that MFM 501 is significant and suitable for further *in vivo* investigations. Our data demonstrated that synthetically-produced intermediate compounds based from natural products may play an important role in the discovery of new anti-infective agents against MRSA. Nevertheless, further pharmacodynamic and *in vivo* toxicology studies are needed to ascertain their potential therapeutic applications.

NOVEL CATIONIC PEPTIDES AND POLYMERS EXHIBIT BACTERICIDAL ACTIVITY AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND MULTI-DRUG RESISTANT *ACINETOBACTER BAUMANNII* CLINICAL ISOLATES TAKEN FROM SKIN INFECTIONS

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The human skin protects the body, acting as an outer barrier against various environmental threats including a vast quantity of microbes. Antimicrobial peptides (AMPs) play the most important role in the cutaneous first line defence, exhibiting broad spectrum antimicrobial activities against potentially harmful pathogens. The increase in multi-drug resistant bacteria causing skin infections and the lack of therapeutic options continues to pose a serious threat for public health. Endogenous AMPs cannot be used directly as antibacterial therapies due to the multiple physiochemical and functional complexities of these ancient immune effector molecules. NovaBiotics has applied a rational drug design approach to re-engineer naturally occurring antimicrobial peptides as clinically and commercially applicable antibacterials. In this study the bactericidal activity of two of these novel compounds was determined. Both therapeutic candidates demonstrated rapid bactericidal activity against two of the leading pathogens of skin infections- methicillin resistant Staphylococcus aureus (MRSA) and Acinetobacter baumannii in nutrient rich and nutrient limiting environments within 1 hour of treatment. The cationic candidates have been demonstrated via transmission electron microscopy (TEM), scanning electron microscopy (SEM) and fluorescent microscopy to uniquely perturb and lyse the cell membrane through electrostatic interactions on both the Gram positive and Gram negative bacterial cell wall, resulting in a rapid bactericidal effect.

USE OF IN VITRO DYNAMIC MODELS TO PREDICT ANTIBIOTIC DOSING THAT PREVENTS DEVELOPMENT OF BACTERIAL RESISTANCE: A REVIEW OF RECENT STUDIES

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The worldwide emergence of antibiotic resistant bacteria threatens the usefulness of available antibiotics, but it might be possible to minimize the selection of resistant organisms by optimizing antibiotic dosing and pharmacodynamics. In vitro dynamic models (IVDM) allow the exposure of growing bacterial cultures to changing concentrations of antibiotics that precisely mimic their human pharmacokinetics. Using IVDM, this laboratory has extensively studied relationships of the enrichment of resistant mutants with antibiotic concentrations. The recently developed concept of the mutant selection window (MSW) [which refers to the antibiotic concentration above the MIC and below the mutant prevention concentration (MPC), the lowest concentration that prevents the selection of first order mutants in vitro], has been tested for the first time in IVDM. Potential predictors of the prevention of the enrichment of resistant mutants, including AUC_{24}/MIC, AUC_{24}/MPC, and time within the MSW (T_{MSW}) have been examined using a newly described area under the bacterial mutant concentration curve ($AUBC_M$). The goal of these studies has been to predict the anti-mutant concentration of antibiotic that might or might not be clinically achievable in human subjects, with an eye towards designing dosing regimens that will optimize bacterial killing and minimize the amplification of resistant mutants.

Recent studies have shown: 1) increased $AUBC_M$ results from longer duration of daptomycin exposure to *Staphylococcus aureus*; 2) slightly higher anti-mutant potentials were found for doripenem than with ciprofloxacin or imipenem against *Pseudomonas aeruginosa;* 3) AUC_{24}/MPC might be a better inter-species predictor of bacterial resistance than AUC_{24}/MIC; 4) moxifloxacin plus docycycline prevents the selection of resistant strains of *Staphylococcus aureus*; 5) the selection of resistant staphylococci by ciprofloxacin is not influenced by the mode of antibiotic administration and is better predicted by AUC_{24}/MPC than AUC_{24}/MIC; 6) doxycycline combinations with linezolid can prevent the enrichment of linezolid resistant *Enterococcus faecium;* 7) T_{MSW} is a good predictor of the amplification of resistant mutants, and our data suggest that plots of T_{MSW} with $AUBC_M$ exhibit a hysteresis loop with more resistant mutants selected when the time above the MPC was 0 than when it was >0. These studies have been performed with many different antibiotic classes against a variety of pathogenic organisms. Studies using IVDM are useful in predicting the optimal dosing regimen that will result in acceptable bacterial killing with minimal selection of antibiotic-resistant mutants, and they can be particularly helpful in the study of antibiotic combinations that will result in enhanced bacterial killing and the prevention of bacterial resistance.

IS A CITRUS ESSENTAIL OIL VAPOUR THE ANSWER TO SURFACE DECONTAMINATION IN THE HOSPITAL ENVIRONMENT?

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Surface contamination in the hospital environment is often associated with the spread of antibiotic resistant bacteria from patient to patient. Lack of sufficient cleaning of surfaces or inappropriate use of disinfectants can also lead to the formation of biofilms on surfaces that are then difficult to remove. The use of a citrus essential oil (EO) based vapour may be the answer to maintaining low levels of antibiotic resistant strains in the hospital environment resulting in reduced cross contamination between patients thus potentially lowering the prescribing rates of antibiotics. The citrus EO vapour has shown to be effective against a range of microorganisms including *Enterococcus* sp., Vancomycin Resistant *Enterococcus* sp.(VRE), *Staphylococcus aureus*, Methicillin Resistant *S. aureus* (MRSA), and *E. coli.* With reductions of VRE and MRSA on stainless steel surfaces after 24hrs being up to a 3 $\log_{(10)}$. The vapour is effective against VRE and MRSA biofilms with a 72% reduction in metabolism of the biofilm and a reduction in coverage of a stainless steel surface of up to 99.5%. The mechanisms of action of the citrus EO vapour has also been explored showing a x40 increase in membrane permeability of the cells, resulting in a loss of intracellular pH, ATP and membrane potential. The main antimicrobial components of the vapour are linalool, citral and β-pinene as determined by a bioautography method and quantified GC-MS. Many of the vapour and spray disinfectants used to decontaminate wards means that patients have to be removed from the environment due to toxicity of the disinfectant such as hydrogen peroxide and ozone, this leads to increased costs to the NHS. The citrus vapour is, however, Generally Recognised as Safe (GRAS) by the Food and Drug Administration (FDA) and can be used in the ward whilst patients are present and still maintain microbial cleanliness of surfaces. In addition, to date there have been no reports of bacterial resistance to EOs.

GENOME SEQUENCING TO IDENTIFY PUTATIVE BACTERIOCIN RECEPTORS

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Most bacteriocins are thought to kill target cells by a receptor-mediated mechanism. It has been shown that the pediocin-like bacteriocins and lactococcin A use the IIC and IID components of the mannose phosphotransferase (man-PTS) as a receptor. However, for most bacteriocins, the identity of the receptor is unknown. In an attempt to identify possible receptors for the two-peptide bacteriocin lactococcin G we have generated 12 independent mutants of *Lactococcus lactis* that are resistant to lactococcin G. The genome of these mutants was sequenced (Illumina system) and compared to the genome of the sensitive wild type stain using the polymorphism discovery algorithm VAAL. As a proof of concept, two lactococcin A-resistant strains were analyzed and both strains contained mutations in the gene encoding either the IIC or the IID component of the man-PTS, the known receptor for this bacteriocin. The genome of all lactococcin G-resistant mutants was identical to the wild type, except for point mutations in or near the gene *uppP*, encoding an undecaprenyl pyrophosphate phosphatase. In nine of the twelve mutants stop-codons or frame shifts were introduced, causing expression of truncated versions of UppP. Two of the mutants contained mutations in putative regulatory regions and the last mutant contained a point mutation leading to a D2N substitution in UppP.
Furthermore, non-sensitive *Streptococcus pneumoniae* became sensitive to lactococcin G when expressing the lactococcal *uppP*, further demonstrating that UppP is involved in sensitivity to lactococcin G. We also show that the related bacteriocin enterocin 1071 (57% sequence identity with lactococcin G) uses the same target receptor.
Our results show that whole genome sequencing of spontaneous bacteriocin-resistant strains may be used as a tool to identify bacteriocin receptor candidates.

ANTIMICROBIAL ACTIVITY OF LACTOFERRICIN PEPTIDES AGAINST MYCOBACTERIUM AVIUM - IMPROVED ACTIVITY OF ARGININE- AND D-AMINO ACIDS-CONTAINING PEPTIDES AND SYNERGISTIC EFFECT WITH ETHAMBUTOL

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Mycobacterium avium causes respiratory disease in susceptible individuals, as well as disseminated infections in immunocompromised hosts, being an important cause of morbidity and mortality among these populations. Current therapies consist of a combination of antibiotics taken for at least six months, with no more than 60% overall clinical success. Furthermore, mycobacterial antibiotic resistance is increasing worldwide, urging the need to develop novel classes of antimicrobial drugs.
One potential and interesting alternative strategy is the use of antimicrobial peptides (AMP). These are present in almost all living organisms as part of their immune system, acting as a first barrier against invading pathogens. The mode of action of AMP is still under debate. Although in most cases they are thought to act primarily by membrane disruption, by a variety of mechanisms, the concomitant presence of internal targets has also been proposed. AMP have the capacity to directly kill bacteria and other microorganisms and, since they act on microbial

membranes, the possibility of their use together with conventional antibiotics as adjunct agents, leading to a synergistic effect, deserves detailed study.
In this context, we investigated the effect of several lactoferrin-derived AMP against *M. avium*. Short peptide sequences from both human and bovine lactoferricins, namely hLFcin1-11 and LFcin17-30, as well as variants obtained by specific amino acid substitutions (arginine by lysines and *vice-versa*), were evaluated. All tested peptides significantly inhibited the axenic growth of *M. avium*, the bovine peptides being more active than the human. Arginine residues were found to be crucial for the display of antimycobacterial activity, whereas the all-D amino acid analogue of the bovine sequence displayed the highest mycobactericidal activity. Interestingly all peptides are much less effective against *M. avium* growing inside macrophages. However, when combined with ethambutol, which alone shows poor activity against *M. avium* growing inside macrophages, there is a synergistic antibacterial effect, suggesting that the AMP increases the access of the antibiotic to the mycobacteria, when they are inside its host cell.
These findings reveal the promising potential of lactoferricins against mycobacteria, and their possible used as adjunct therapy, thus opening the way for further research on their development and use as a new weapon against mycobacterial infections.

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CHARACTERIZATION OF MEMBRANE-ACTIVE ANTIMICROBIAL PEPTIDES DERIVED FROM MSI-78

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Antimicrobial peptides (AMP) are a class of broad-spectrum antibiotics known by their ability to disrupt bacterial membranes and their low tendency to induce bacterial resistance, arising as excellent candidates to fight bacterial infections. In this study we aimed at designing short 12-mer AMP, derived from MSI-78 (22 residues), a highly effective and broad spectrum synthetic AMP, by truncating the peptide at the *N*- and/or *C*-termini while spanning the MSI-78 sequence with 1 amino acid (aa) shifts [1,2]. These designed peptides were evaluated regarding antimicrobial activity against selected gram-positive *Staphylococcus* strains and the gram-negative *Pseudomonas aeruginosa (P. aeruginosa)*.
In general most of the short 12-mer peptides tested showed a reduction in antimicrobial activity, an effect that was more pronounced for gram-positive *Staphylococcus* strains. However, the 12-mer peptide CEM1 (GIGKFLKKAKKF) arose as a good candidate to fight *P. aeruginosa* infections, as it displays antimicrobial activity against this strain and selectivity, with negligible toxicity to mammalian cells even at high concentrations. Interestingly, CEM1 and a highly similar peptide differing by only one aa-shift (CEM2: IGKFLKKAKKFG), showed a remarkably contrasting AMP activity. These two peptides were chosen for a more detailed study regarding their mechanism of action, using several biophysical assays and simple membrane models that mimic the mammalian and bacterial lipid composition.
We found a direct correlation between peptide helicity and antimicrobial activity and propose a mechanism of action that supports an antibacterial action based on bacterial membrane lysis and consequent bacterial cell death.
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SYNERGISITC PHOTODYNAMIC ANTIMICROBIAL ACTIVITY OF METHYLENE BLUE AND CHITOSAN AGAINST *Helicobacter pylori 26695*

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Background: Photodynamic therapy (PDT) is a method for killing cells (bacterial, fungal and cancer cells) or virus using photosensitizers (PS) and light of various wavelengths. *In vitro* PDT using endoscopic light against *H. pylori* was effective at a concentration of 0.2 mg/mL of MB.(J Photochem Photobiol 2010;101:206) The purpose of this study was to increase the effect of photodynamic modality against *H. pylori* by addition of chitosan to MB and to decrease MB toxicity.
Methods: The bactericidal effect was measured by counting viable cells after PDT. The degree of damage to DNA was confirmed using alkaline gel electrophoresis. Cellular DNA damage was demonstrated by ethidium bromide monoazide–quantitative polymerase chain reaction.
Results: In the groups treated with either 0.04 mg/mL MB alone or 0.02 mg/mL MB with endoscopic light for 15 min, viable cells were decreased approximately tenfold. The group treated with 0.04 mg/mL of MB with light, showed more bactericidal activity than 0.02 mg/mL MB treatment. By 0.05% chitosan pre-treatment followed with 0.04 mg/mL of MB and light irradiation, viable cells were decreased 107-fold. The DNA damage caused by PDT as demonstrated by alkaline gel electrophoresis was greater in the MB plus chitosan-treated group than in control and MB-treated groups. Cellular DNA damage demonstrated byEMA-qPCR was also greater in the group treated with MB plus chitosan than in the MB-treated group. Conclusion: The bactericidal effects with PDT using MB were increased with the concentration of photosensitizer and chitosan treatment, peculiarly before endoscopic light irradiation.

DEVELOPMENT OF LEVOFLOXACIN-LOADED HYALURONIC ACID NANOHYDROGELS FOR THE TREATMENT OF INTRACELLULAR BACTERIAL INFECTIONS.

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Purpose. Numerous antimicrobial drugs have been reported to kill or inhibit the growth of microbes such as bacteria. Even though the therapeutic efficacy of these drugs has been established, often their use fails for intracellular infections. The aim of this project was to entrap levofloxacin (LVF), one of the most widely available fuoroquinolone antibiotics, into hyaluronic acid-cholesterol nanohydrogels (HA-CH NHs) in order to facilitate the entrance of the drug inside the cells and to treat intracellular infections caused by Staphylococcus aureus and Pseudomonas aeruginosa.
Methods. To obtain NHs, cholesterol was linked to HA chains by a bifunctional spacer, 4-bromobutyric acid, with a double step reaction. HA-CH amphiphilic polymer was dissolved in N-methyl-pyrrolidone (NMP) and co-precipitated in a LVF aqueous solution. The obtained LVF-loaded NHs (NH-LVF) were purified through dialysis against water in order to eliminate the organic solvent and the unloaded drug. HA-CH NH-LVF suspension was sterilized through autoclaving process and freeze-dried in the presence of dextrose as cryoprotectant. The MIC values of NHs-LVF for Staphylococcus aureus and Pseudomonas aeruginosa was investigated and compared to that of free LVF. The intracellular antimicrobial activity of NH-LVF and free LVF was also determined on infected HeLa cell line.
Results. Amphiphilic HA-CH polymer, dissolved in a suitable organic solvent, can be precipitated drop by drop in LVF solution, spontaneously forming self-assembled NHs entrapping the antibiotic (drug loading 15% ±2% w/w, defined as the ratio between the amount of the encapsulated LVF and the initial amount of the drug). NH-LVF suspension in water was

characterized by narrow distribution size (160±20 nm). In order to obtain NH-LVF sterile powder, the suspension was added to dextrose solution (1% w/v), sterilized by autoclaving and freeze-dried. The powder was re-suspended in water and the NH-LVF was tested on Staphylococcus aureus and Pseudomonas aeruginosa strains. NH-LVF showed almost the same MIC values, compared to those of free LVF. MIC values on Staphylococcus aureus were 175 ± 78 ng/ml for free LVF and 104 ± 58 ng/ml for NHs-LVF in aqueous suspension. MIC values on Pseudomonas aeruginosa were 940 ± 0 ng/ml for free LVF and 557 ± 78 ng/ml for NHs-LVF in aqueous suspension.
Experiments performed on infected cells demonstrated that, when entrapped in NHs, LVF has a much higher intracellular activity then free LVF.
Conclusions. Intracellular infections are very difficult to treat. By loading the LVF in HA-CH NHs, it is possible to overcome such limitations, increasing the antibiotic cellular uptake and promoting the levofloxacin therapeutic efficacy.

ANTIMICROBIAL PEPTIDES FROM WILD BEE VENOM AGAINST MICROBIAL BIOFILMS

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In natural habitat, microbes tend to adhere to biotic and abiotic surfaces and grow in physiologically distinct form, the biofilm. Due to increased use of implanted devices in medicine, the incidence of biofilm-associated illnesses is growing. However, biofilm infections are not only limited to implanted devices but they can also be associated with pneumonia in cystic fibrosis patients, chronic wounds, chronic otitis and catheter-associated infections. Biofilm represents a unique adaptation mechanism with lower growth rate and increased drug resistance. Eradication of biofilm is more challenging than in case of planktonic cells. Most of currently used drugs act well against actively growing cells but are less efficient against biofilm. There is an urgent need for development of new drugs to fight biofilm infections. Antimicrobial peptides represent one of the promising groups with unique mechanism of action against biofilm and actively growing bacteria and fungi.
Here we show the impact of new synthetic antimicrobial peptides isolated from the venom of wild bee Hylaeus signatus and Halictus sexcinctus on biofilm and planktonic cells. The peptides were originally isolated in our laboratory and belong to the category of short linear alpha-helical peptides. We subjected to our studies a representative of major human pathogens from Gram positive (Staphylococcus aureus) and negative (Pseudomonas aeruginosa) bacteria as well as fungi (Candida albicans). Furthermore we focused our objective on two distinct ways how to control biofilm – prevention (growth of biofilm) and treatment (pre-formed biofilm).
We observed growth kinetic for planktonic cells in the presence of various concentrations of peptides and determined minimal inhibition concentrations (MIC); some of our peptides show excellent potency against multi-drug resistant microbes. In case of pre-formed biofilm, we used metabolic activity as an assessment of community fitness and growth viability for survival determination. We used light microscopy for fungi and studied morphological changes in biofilm development in the presence of peptides. We also studied toxicity of the peptides towards human blood cells.
Our peptides were able to effectively inhibit growth of planktonic cells in concentrations below 20 μmol/l. Some of the peptides not only completely inhibited metabolic activity of pre-formed biofilm at concentrations not exceeding five times MIC for planktonic cells but they also kill the cells in the bacterial and fungal biofilm. We also observed changes in the morphology of Candida albicans in the presence of our peptide; the hyphal structures were formed later with increasing concentration of the peptide. The haemolytic activity of some of the peptides was negligible.
The ability to kill individual cells in biofilm represent valuable potential for medicine and therefore our peptides represent promising candidates as future antimicrobial agents.

Acknowledgement: This work was supported by research project RVO 61388963 of the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic.

MANUKA HONEY ENHANCES THE EFFICACY OF TOPICAL ANTIMICROBIAL AGENTS WHEN USED AGAINST BIOFILM CULTURES OF STAPHYLOCOCCUS EPIDERMIDIS IN VITRO

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Background: Staphylococcus epidermidis (S. epidermidis) is a common commensal bacteria that can cause infection, usually in conjunction with medical devices such as catheters. Successful treatment of these infections has become increasingly difficult with increased bacterial resistance to antibiotics limiting treatment options. Manuka honey has been shown to inhibit several species of pathogenic organism, and more recently combinations of antibiotics and honey have revealed synergistic activity against various bacterial species. This study aimed to test whether manuka honey and selected antibiotics were synergistic for biofilms of S. epidermidis.

Methods: The antibiotics tested were: fusidic acid, metronidazole, and mupirocin (Sigma, UK) at concentrations ranging from 512 mg/l – 0.0078 mg/l, and manuka honey (Medihoney, Comvita, NZ) was also tested.
The European Committee on Antimicrobial Susceptibility Testing guidelines were used to determine minimum inhibitory concentration and minimum biofilm eradication concentration of the antimicrobial agents using micro broth dilution, for S. epidermidis (ATCC 12228).

Checkerboard assays were used to detect synergistic combinations, biomass was measured with crystal violet and viability with resazurin. The fractional inhibitory concentrations were then calculated. Tests were done in triplicate.

Results: Combining each of the antibiotics with manuka honey resulted synergistic activity in all cases. The MIC and MBEC of all three antibiotics was reduced when combined with below the MIC amounts of manuka honey. All FIC and FBECI values were less than 0.5.

Discussion: Combining antibiotics and manuka honey led to improved activity of antibiotics against S. epidermidis. Honey could potentially be used to increase efficacy of topical antibiotic therapy in clinical practice.

THE EFFECT OF CECROPIN A-MELITTIN ANTIMICROBIAL PEPTIDES ON POPE/POPG MODEL MEMBRANES – A BIOPHYSICAL APPROACH USING SANS AND SAXD

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New strains of infectious agents resistant to known antibiotics are increasingly appearing, an alarming situation as the antibiotics discovery is not keeping pace with this reality. In recent years, antimicrobial peptides (AMPs) have emerged as an interesting alternative. They are thought to act mainly by membrane disruption, and the proposed mechanisms for antimicrobial activity involve an electrostatic attraction and binding of the peptide to the surface of the lipid bilayer, with consequent destabilization of the membrane and/or translocation of the peptide to the cytoplasm. Only recently the importance of nonlamellar lipid phases induced by AMPs has been addressed as a possible mode of action, and it may provide a reasonable explanation for the destabilization of the lipid bilayer.
In this work we have characterized the interaction of three AMPs, a cecropin A-melittin hybrid (CA(1-7)M(2-9)) (CAM) and two lysine Nε-trimethylated analogs (K6 and K7), with model membranes of POPE/POPG=3:1 mol/mol, considered to be a good model for bacterial membrane. Our previous study of CAM and its lysine N-trimethylated analogs (K6 and K7) have shown a strong interaction with negatively charged liposomes (bacterial cell model), as derived from DSC and fluorescence spectroscopy.

Small Angle X-ray Diffraction (SAXD) results show that the addition of peptides to POPE/POPG(3:1) membrane lowers dramatically its periodicity (d) when the system goes from gel (d~11 nm) to liquid-crystalline state (d~5.3 nm). The smaller periodicity observed in the presence of peptides is similar to the one measured for pure POPE in the same experimental conditions. This indicates a strong interaction but does not provide a clear picture about the mechanism of membrane destabilization. We hypothesized the presence of mixed POPG-CAM micelles, formed as a result of induced charge segregation in POPE/POPG bilayer upon peptide interaction and insertion, leading its disruption.
Small Angle Neutron Scattering (SANS) employing contrast variation technique was used to characterize fully the peptide/lipid membrane interaction. The effect of CAM was studied at three lipid:peptide molar ratios, L:P=25:1, 17:1 and 10:1 and samples were measured at selected temperatures. The experiments show a structural reorganization of the membrane, however they do not confirm unequivocally the presence of lipid/peptide micelles.
These findings indicate that the pathway of CAM antimicrobial activity is not explained by membrane micellization or formation of non-lamellar structures (such as hexagonal or cubic phases). The obtained results support the so called "carpet model" as a possible mechanism of antimicrobial activity for these peptides.

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TACKLING THE WEAK LINK: LYSINE REPLACEMENT ENHANCES STABILITY OF POTENT ANTI-MYCOBACTERIAL PEPTIDES

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Objectives

The rapid emergence of multi-drug resistant tuberculosis (TB) and the lack of effective therapies have prompted the development of compounds with novel mechanisms of action to tackle this growing public health concern. Recently, we reported on a series of short amphipathic ?-helical antimicrobial peptides (AMPs), comprising the backbone sequence (LLKK)2, with the ability to kill susceptible and drug-resistant M. tuberculosis. These AMPs were also shown to prevent the development of drug resistance, highlighting their potential utility in combating multidrug-resistant TB. Poor in vivo stabilities, however, have largely limited the clinical development of peptide therapeutics to topical applications. In this study, the effectiveness of unnatural amino acid substitutions as a strategy to enhance peptide resistance to proteolytic cleavage was investigated.

Methods

Synthetic peptides were designed by replacing a single lysine residue in each LLKK repeat unit to obtain peptides with the primary sequence I(LLKX)2I and I(LLXK)2I, where X is either ornithine (Orn), diaminobutyric acid (Dab) or diaminopropionic acid (Dap). Anti-mycobacterial activities were quantified by determination of the minimum inhibitory concentration (MIC) using standard broth microdilution method in M. smegmatis. The stability of the peptides to degradation following treatment with the serum protease trypsin also was evaluated. Killing efficiency of the peptides was measured at 0, 0.5, 1 and 2x MIC concentrations.

Results

Substitution of unnatural amino acids into the LLKK peptide backbone preserved anti-mycobacterial activity against M. smegmatis except for I(LL-Orn-K)2I, which was rendered ineffective. MALDI-TOF MS analysis revealed that this peptide was unstable with numerous peaks observed in the spectrum. Following trypsin treatment, I(LLKK)2I lost its activity against

M. smegmatis, suggesting that the peptide had undergone proteolytic degradation. All other peptides except I(LL-Orn-K)2I effectively inhibited bacterial growth, with three peptides recording a two-fold increase in MIC. Dab-substituted analogues displayed the highest stability towards proteolytic degradation with an MIC of 62.5 μg/ml, both in the presence and absence of trypsin. Synthetic peptides exhibited killing efficiencies of 100% at 2x MIC and >99% at MIC.

Conclusion

The degradation of the parent peptide, I(LLKK)2I, was successfully prevented by replacing a single lysine residue in each repeat unit with Orn, Dab or Dap. Furthermore, the anti-mycobacterial activities of the peptides were not compromised. These findings underscore the potential of this strategy to enhance peptide stability to serum proteases such as trypsin.

ANTIMICROBIAL ACTIVITY OF LACTOFERRICIN PEPTIDES IN SYNERGY WITH PLANT EXTRACTS FOR THE TREATMENT OF OTITIS EXTERNA IN DOGS

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Otitis externa (OE) is the most common disease of the ear canal in dogs and it has a multifactorial etiology (e.g. atopic dermatitis, food allergy, etc). Microorganisms most frequently isolated are *Staphylococcus intermedius group (SIG), Pseudomonas aeruginosa, Proteus vulgaris and Malassezia pachydermatis.*

Traditionally, OE treatments include topical and/or systemic use of antibiotics, antifungals and glucocorticoids. Because of the rising antibiotic-resistance phenomenon, development of alternative antimicrobial agents is encouraged. Bovine lactoferricin is a multi-functional peptide derived from proteolytic cleavage of bovine lactoferrin. It has proved antibacterial, antifungal and immunostimulatory activity.

The aim of this study was to evaluate the action of a patent pending mixture (LAS™, filed with number MI2014A 00130 on 24/09/2014) of lactoferricin peptides associated with two plant extracts with antinflammatory effects, in dogs with erythematous ceruminous otitis externa and bacterial or *Malassezia* spp. overgrowth.

The product was tested in 10 dogs, selected according to the good clinical practice, showing recurrent erythematous and ceruminous OE. Treatment consisted in topical daily administration of the mixture. The patients were submitted to the clinical-instrumental evaluations during the inclusion examination day and at days 7, 14 and 21 of treatment.

Each visit included otoscopy and evaluation of the ear condition using a modified CADESI system and cytology of the ear, performed with a sterile swab. The obtained samples were stained by Diff Quick® (Diff-Quik, Medion Diagnostics AG, Düdingen, Switzerland) and examined at 40/HPF for the count of keratinocytes, bacteria and *Malassezia* spp.

The software used was SPSS (version 19; SPPS Inc., Chicago, IL, USA). Data obtained were analyzed with Shapiro-Wilk, Kruskal-Wallis and Friedman tests. A P value < 0.05 was considered statistically significant.

Results underlined a statistically significant reduction of both ear canal lesions and CADESI score. Moreover, cytological examinations showed a reduction in the number of keratinocytes, bacteria and *Malassezia* spp.

The topical product (LAS™) was effective to treat and control bacterial and/or *Malassezia* overgrowth in dogs with erythematous and ceruminous otitis externa. It showed antibacterial and antifungal activity, in addition to ceruminolytic action.

AN INVESTIGATION OF THE DOUBLE AND TRIPLE SYNERGISTIC ANTIMICROBIAL INTERACTIONS BETWEEN LITSEA, ROSEWOOD AND CLOVE ESSENTIAL OILS AGAINST ACNE VULGARIS-ASSOCIATED BACTERIA *PROPIONIBACTERIUM ACNES* AND *STAPHYLOCOCCUS EPIDERMIDIS*

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Acne vulgaris, a chronic inflammatory disease of the pilosebaceous unit, affects approximately 80% of 11-30 year olds. Overgrowth of *Propionibacterium acnes* is thought to drive chemotactic and inflammatory mediator production whilst *Staphylococcus epidermidis* is thought to be a secondary coloniser, potentiating inflammation in the pilosebaceous unit. Antibiotics are often prescribed for acne as they control *P. acnes* proliferation and inflammation. However, decreased antibiotic sensitivity of *P. acnes* has been observed in recent years, which is often associated with therapeutic failure, providing a need for alternative antimicrobial treatments. Essential oils (EOs) are known to possess antimicrobial activity, and some to have synergistic activity when used in combination. This study aims to determine the synergistic, antagonistic or additive interactions between double and triple combinations of litsea, rosewood and clove EOs against *P. acnes* and *S. epidermidis* in order to evaluate their viability as an alternative acne treatment. Preliminary screening of the antimicrobial activity of 12 essential oils was carried out using the disc diffusion method. Litsea, rosewood and clove were investigated further due to their antimicrobial activity against both test species. Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were then determined using a broth dilution method. Synergistic interactions between combinations of 2 and 3 EOs were determined using a modified 3D checkerboard method with concentrations ranging from 0.25% to 0.01%. The Fractional Inhibitory Concentration (FIC) was then calculated to determine the synergistic, antagonistic or additive interactions between the EOs. Double combinations of EOs possessed synergistic interactions against *S. epidermidis* at lower concentrations (0.01-0.03%) and additive interactions at higher concentrations (0.06-0.125%). Only a combination of 0.03% rosewood with 0.06% litsea was synergistic against *P. acnes.* Triple combinations of 0.01% litsea and clove with either 0.01% or 0.03% rosewood were synergistic against *S. epidermidis* and 0.06% litsea with 0.01% clove and rosewood was synergistic against *P. acnes.* All other triple combinations were additive. Results show litsea, rosewood and clove EOs possess concentration-dependent synergistic interactions in both double and triple combinations. Triple combinations had the greatest antimicrobial effect, lowering concentrations of EOs required to inhibit the growth of acne-associated bacteria. The use of combinations of EOs may, therefore, be a viable alternative treatment to antibiotics for acne vulgaris.

IDENTIFICATION OF BACTERIOCIN RESISTANCE-INDUCING MUTATIONS BY WHOLE GENOME SEQUENCING WITHOUT A REFERENCE GENOME: THE PUTATIVE RECEPTOR OF PLANTARICIN JK

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Earlier we have identified putative bacteriocin receptors by whole genome Illumina sequencing of bacteriocin-resistant mutants from sensitive strains and use of the VAAL polymorphism discovery algorithm to compare the reads to a reference database genome. For the indicator strain used with plantaricin JK, *Weissella viridescens*, no reference genome is available. We circumvented this problem by assembling Illumina reads to obtain a preliminary reference genome, and then used VAAL to compare reads from 11 plantaricin JK-resistant mutants with the reference. We ended up with a genome sequence consisting of 243 contigs ranging in size from 70,000 to 300 bp and a total length of 1.59 Mbp. After removal of putative false positives by excluding differences appearing in reads from the wild-type and in all or most of the mutants, each of the mutant strains showed 1-7 differences compared to the reference contigs. All plantaricin JK-resistant mutants carried point mutations in a gene coding for a protein belonging to the APC superfamily of amino acid/polyamine/organocation transporters. Thus this protein emerges as a putative membrane receptor for plantaricin JK.
We recently obtained a more complete assembly of *Weissella* viridescens by assembling long reads from a PacBio instrument to one single 1.6 Mbp contig. When this improved assembly was used as the reference genome in a new VAAL analysis of the Illumina reads, the number of differences between the reference genome and the reads from the sensitive wild-type strains was reduced to zero, while the mutant strains contained 1-8 differences compared to the reference. All mutations in the APC family gene were verified. In conclusion, we show that even without a high-quality reference genome, VAAL can be used to identify putative bacteriocin receptors, as long as the number of mutant genomes analysed is sufficient to allow identification and exclusion of false positives.

ANTIMICROBIAL PEPTIDES FROM WILD BEE VENOM AGAINST MICROBIAL BIOFILMS

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In natural habitat, microbes tend to adhere to biotic and abiotic surfaces and grow in physiologically distinct form, the biofilm. Due to increased use of implanted devices in medicine, the incidence of biofilm-associated illnesses is growing. However, biofilm infections are not only limited to implanted devices but they can also be associated with pneumonia in cystic fibrosis patients, chronic wounds, chronic otitis and catheter-associated infections. Biofilm represents a unique adaptation mechanism with lower growth rate and increased drug resistance. Eradication of biofilm is more challenging than in case of planktonic cells. Most of currently used drugs act well against actively growing cells but are less efficient against biofilm. There is an urgent need for development of new drugs to fight biofilm infections. Antimicrobial peptides represent one of the promising groups with unique mechanism of action against biofilm and actively growing bacteria and fungi.

Here we show the impact of new synthetic antimicrobial peptides isolated from the venom of wild bee *Hylaeus signatus* and *Halictus sexcinctus* on biofilm and planktonic cells. The peptides were originally isolated in our laboratory and belong to the category of short linear alpha-helical peptides. We subjected to our studies a representative of major human pathogens from Gram positive (*Staphylococcus aureus*) and negative (*Pseudomonas aeruginosa*) bacteria as well as fungi (*Candida albicans*). Furthermore we focused our objective on two distinct ways how to control biofilm – prevention (growth of biofilm) and treatment (pre-formed biofilm).

We observed growth kinetic for planktonic cells in the presence of various concentrations of peptides and determined minimal inhibition concentrations (MIC); some of our peptides show excellent potency against multi-drug resistant microbes. In case of pre-formed biofilm, we used metabolic activity as an assessment of community fitness and growth viability for survival determination. We used light microscopy for fungi and studied morphological changes in biofilm development in the presence of peptides. We also studied toxicity of the peptides towards human blood cells.

Our peptides were able to effectively inhibit growth of planktonic cells in concentrations below 20 μmol/l. Some of the peptides not only completely inhibited metabolic activity of pre-formed biofilm at concentrations not exceeding five times MIC for planktonic cells but they also kill the cells in the bacterial and fungal biofilm. We also observed changes in the morphology of *Candida albicans* in the presence of our peptide; the hyphal structures were formed later with increasing concentration of the peptide. The haemolytic activity of some of the peptides was negligible.

The ability to kill individual cells in biofilm represent valuable potential for medicine and therefore our peptides represent promising candidates as future antimicrobial agents.

Acknowledgement: This work was supported by research project RVO 61388963 of the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic.

THE REAL ROLE OF OZONATED AUTOHEMOTHERAPY IN INFECTIONS

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It is unfortunate that today antibiotic therapy has become less effective in both human and animal infections. Therefore, the search for alternatives to antibiotics has led to the evaluation of the use of substances with intrinsic disinfectant properties. Gaseous ozone is a potent oxidant with antibacterial, antimycotic, antiviral as well as antiparasitic activity. Additionally, ozone is able to pierce biofilms in which many microbes hide to protect themselves from other antimicrobials. Moreover, ozone does not create resistant microrganisms. Various therapeutic protocols have been offered for the management of sepsis, among which ozonated autohemotherapy (O-AHT). Such an adjuvant therapeutic technique can be summarized as follows: 150-200 ml of citrate-anticoagulated blood are withdrawn from a patient and they are

collected in a 500 ml sterile glass bottle. Immediately, an equivalent volume of a gaseous mixture of oxygen/ozone (~95%O_2/~5%O_3) is added through a sterile needle in the bottle. At this point, the blood is mixed for about 1 min with the gas phase. Successively, the so-oxygenated-ozonated blood is intravenously re-infused in the donor patient giving rise to an auto-transfusion. In detail, in the case of an infectious disease the concentration of ozone added to the blood can range from an initial 20 μg/ml (3-4 mg) up to 60 μg/ml (9-12 mg) of ozone per ml of blood in successive infusions (2-3 infusion/wk). But what really happens when the gaseous ozone comes into direct contact with the blood, like in the O-AHT? Please note that ozone has both instantaneously and fully reacted with blood components and it is not present as is. In other words, its anti-infective power during parenteral systemic administration is lacking because of the ozone neutralization by the antioxidant capacity of the biological fluids [1,2]. Therefore, its therapeutic contribution in the case of systemic infections is not due to a direct mechanism of action. But if it is the case, how is O-AHT useful in systemic infections? Concisely, leucocytes are activated by ozone via NFκB, causing both a phagocytosis enhancement as well as the release of γ-interferon, IL-2 and TNFα in the patient [3]. For comprehensiveness' sake, an ozone-based unspecific immune-modulator approach has been widely used during the last two decades for treating viral infections. It is the so-called minor O-AHT, which briefly consists of withdrawing 5 ml patient's own blood to be immediately and vigorously mixed for about 1 min with an equal volume of oxygen-ozone gaseous mixture at an ozone concentration ranging between 80 and 100 μg/mL of gas per mL of blood. Such an ozonated blood, including the foam, is promptly injected into the gluteus muscle without the need of any anaesthetic [4].
In conclusion, O-AHT does not possess a specific antibacterial activity at the systemic level. On the other hand, it may activate the immune system against the bacterial infections, strengthening the common antibiotic therapy. A number of O-AHT treatments have been already done in patients as a support in infection diseases and in the absence of side effects.

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THE PROPER USE OF OZONE AND OZONE DERIVATIVES IN TOPICAL INFECTIONS

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Cutaneous and mucosal infections are very frequent and represent a considerable distress for the patients and a great economical burden for Health Authorities. Specifically: i) elderly people or patients with either diabetes or/and atheroschlerosis easily undergo formation of ulcers, bed sores, which never heal due to dismetabolism, invalidity and tissue hypoxia; ii) skin lesions due to trauma or burns may easily degenerate into chronic wounds due to unattended bacterial infections; iii) the vaginal infections sustained by Chlamidia, Candida and Trichomonas are also increasing all the time, particularly in youngsters due to poor hygienic and unprotected sexual intercourses; iv) a number of mucosal infections due to other pathogens as the fastidious aphthous ulcers in the mouth as well as the typical dental caries, periodontal disease, and peri-implantitis; v) the formation of ragadis and fistulae in the rectal mucosa; vi) ophthalmic infections. Valid antibiotic treatments are parenterally and/or topically used. Unfortunately, with time, pathogens may become drug-resistant. By way of example, it has been recently observed that repeated exposure of conjunctival flora to ophthalmic antibiotics selects for resistant strains, as well as repeated use of ophthalmic antibiotics after intraocular injection promotes the emergence of antimicrobial resistance [1]. Moreover, conventional drugs occasionally procure side-effects which limit the compliance. Another drawback is represented by their cost, which compromise their use or their availability in poor Countries.

Historically, since 1916 ozone has been used to treat necrotic wounds, especially if due to anaerobic bacteria Starting from these years, the disinfectant value of ozone has been increasingly appreciated. It is realized that ozone, under various formulations, can display a cleansing effect and act as a potent disinfectant able to practically kill all pathogens present in the skin and mucosal surfaces. Furthermore, the decomposition of ozone, by increasing the availability of oxygen in the ischemic or inflamed tissues, has the additional advantage to improve the local metabolism and the proliferation of tissues, essential for the eventual mucosal or/and cutaneous healings. Recently, in several Countries such as Germany, Italy, Greek, Turkey, Russia and Cuba the possibility of using ozone and ozone derivatives as anti-infective in veterinary and human medicine has been evaluated. In detail, ozone is being appreciated, either as a gas by means of specific medical devices for the application, or as ozonated water, or else as ozonated natural matrices in a variety of infections, trophic ulcers, burns, cellulitis, abscesses, anal fissures, decubitus in paralytic patients, fungal diseases, gingivitis, peritonitis and vulvovaginitis [2].
It should be noted that the application of ozone and ozonated derivatives, mainly ozonated oils, should be preceded by careful cleaning and washing of the biological exudates present in wounds and ulcers [3]. It seems also useful to have a series of ozonated oils characterized by both peculiar unsaturation degree and gradations of peroxide value to be used on wounds that are highly contaminated with bacteria mixed with dead cells. In recent years, a rapid increase in ozonated oil sales through the internet, with dozens of companies and web sites has been observed. However, the quality and efficacy of these products are divisive. In fact, very few are quality consistent, and contain sufficiently ozonated oils in terms of peroxidation level, iodine value, acidity value, viscosity, density and colour variations, just to name a few.
Eventually, another drawback is represented by the correct application modality according to the residence time. In this regard, a correct formulation depending on both the site of application and the type of infection is basic.

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SAFETY ASSESSMENT OF *ENTEROCOCCUS* SPP. ISOLATED FROM RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND ITS AQUATIC ENVIRONMENT AS POTENTIAL FISH PROBIOTICS

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Aquaculture is the fastest-growing food-producing sector worldwide contributing to the increasing demand for food of aquatic origin. Although in the last years, fish farming activity has expanded, diversified and intensified, the emergence of a large variety of pathogens represents a major limiting factor for this sector. The extensive use of antibiotics has been associated with antibiotic resistance development in fish pathogens. In this respect, the use of Lactic Acid Bacteria of aquatic origin as probiotics constitutes an alternative strategy to the antibiotic treatment for disease control in the aquaculture farming. Therefore, several *Enterococcus* spp. strains are currently used as probiotics in human and animal health. The main objectives of this work were (i) the safety assessment of 64 *Enterococcus* spp. strains isolated from the complete

biological life cycle of farmed rainbow trout (*Oncorhynchus mykiss*), and its aquatic environment (vegetation, tank waters and their commercial feeding) and (ii) the evaluation of their antimicrobial activity against the main Gram-negative and Gram-positive fish pathogens.
A total of 64 enterococcal strains were identified to the species level by PCR, using primers and conditions for the different enterococcal species. The taxonomical identification was confirmed by DNA sequencing of superoxide dismutase (*sodA*) amplified by PCR. Safety of enterococci was first evaluated by analysis of hemolysin and gelatinase production and by PCR-detection of genes encoding virulence factors. Antibiotic susceptibility patterns were determined by a disk-diffusion method and the presence of transferable antibiotic resistance genes was determined by PCR. Antimicrobial activity was determined by a stab-on-agar test against the most relevant fish pathogens (*i.e.*, *Streptococcus iniae*, *Lactococcus garvieae*, *Yersinia ruckeri*, *Vibrio campbellii* and *Aeromonas salmonicida*). The presence of several common enterococci bacteriocin-encoding genes was investigated by PCR.
The taxonomic identification to the species level of the 64 enterococcal strains showed that 27 strains (42.2%) were identified as *Enteroccus faecium*, 23 strains (35.9%) as *E. hirae*, 8 strains (12.5%) as *E. faecalis*, 3 strains (4.7%) as *E. durans* and 3 strains (4.7%) as *E. casseliflavus*. The production of gelatinase and hemolysin was phenotypically detected in 1 and 5 strains, respectively. The virulence factors *gelE* (30 strains, 46.9%), *efaAfs* (11 strains, 17.2%), *agg* (1 strain, 1.6%) and *hyl* (1 strain, 1.6%) were detected. A total of 51 strains (79.7%) showed phenotypic resistance to, at least, one antibiotic: erythromycin (19 strains, 37.3%), tetracycline (12 strains, 23.5%), ciprofloxacin (8 strains, 15.7%), quinupristin-dalfopristin (5 strains, 9.8%), vancomycin (4 strains, 7.8%), chloramphenicol (2 strains, 3.9%) and streptomycin (1 strain, 1.9%). Moreover, 25 out of 64 strains (39.1%) harboured, at least, one antibiotic resistance gene: *erm*(B) (14 strains, 56%), *tet*(M) (6 strains, 24%), *tet*(S) (4 strains, 16%), *tet*(K) (3 strains, 12%), *tet*(L) (3 strains, 12%), *tet*(T) (1 strain, 4%), *van*C2 (3 strains, 12%) and *aad*(E) (1 strain, 4%). A total of 48 strains (75%) showed direct antimicrobial activity against, at least, one of the fish pathogens tested. From these strains, 24 (50%) harboured, at least, one bacteriocin-encoding gene: *entP* (20 strains, 83.3%), *entL50A* and *entL50B* (12 strains, 50%), *entJSA* and *entJSB* (11 strains, 45.8%), *hirJM79* (4 strains, 16.7%), *entSE-K4* (3 strains, 12.5%), *entQ* (3 strains, 12.5%) and *entA* (1 strain, 4.2%).
In conclusion, a total of 17 (26.6%) enterococcal strains that did not harbour any antibiotic resistance nor virulence were considered as safe, including six strains (35.3%) that showed antimicrobial activity against, at least, one fish pathogen and harboured, at least, one bacteriocin-encoding gene. These results reveals that the rainbow trout and its aquatic environment are an interesting and appropriate source for the isolation of enterococcal strains with a potential application as probiotics for a sustainable aquaculture. However, *in vivo* safety assessment must be addressed before proposal of these strains application as rainbow trout probiotics.

Day 3: Modulating the Immune System

Invited Speakers Abstracts

Enhancing Host Resistance against Lethal Infections

Professor Roger M. Loria, Virginia Commonwealth University Medical Center, Richmond, Virginia, USA
This report illustrates that the androstene hormones are able to upregulate the host immune response to a level that enables the host to resist lethal infection by gram positive and gram negative bacteria as well as viruses. In vivo, the androstenes increase the levels of the Th1 cytokines such as IL-2, IL-3, and IFN. Similar to hydrocortisone, they suppress inflammation, but do not suppress immunity.The agents have a role in the maintenance of the Th1/Th2 balance and immune homeostasis.

Antimicrobial peptides from Echinoderms as antibiofilm agents

Dr Domenico Schillaci, Lecturer, University of Palermo, Palermo, Italy

Antimicrobial peptides (AMPs) represent the first line of defence against pathogens in several organisms. Recently, it was demonstrated that the AMPs from immune cells of Echinoderms are active against staphylococcal and Pseudomonas aeruginosa biofilms. The discovery of novel peptides in Echinoderms represents a good starting point to design new synthetic derivatives with modified chemical-physical properties, with the aim to improve their antimicrobial activity against pathogens and their pharmaceutical potential. The data show a great potential of AMPs for developing novel therapeutic agents that are either alternative or complementary to conventional antibiotic therapy to combat multi-resistant pathogens.

HBD3 regulates matrix metalloproteinase production in human myeloid dendritic cells exposed to Porphyromonas gingivalis hemagglutinin B (HagB)

Professor Kim Alan Brogden, The University of Iowa, Iowa City, USA

Matrix metalloproteinases (MMP) are zinc- or calcium-dependent proteinases involved in the normal maintenance of the extracellular matrix. When elevated, MMPs degrade matrix components contributing to tissue destruction in infected periodontal sites. Recently we found that HBD3 regulates MMP responses in dendritic cells exposed to Porphyromonas gingivalis hemagglutinin B (HagB). HagB induced the expression of MMPs and HBD3 induced a unique bidirectional modulation of the MMP response after HBD3 treatment. Thus, HBD3 can alter the profile of HagB-induced MMPs and such a finding may have importance in the pathogenesis of periodontal disease.

Antibiotic therapy corroborated by selected immunomodulating beta-blockers

Dr. Karin Peuschel, CNP, Switzerland

Pharmacological strategies that target activation of the immune system may lead to an improved elimination of foreign antigen like for example bacteria, that is they are considered to have an indirect antibiotic effect acting not directly at the level of the infective agent. The non-selective beta-blocker Propranolol has been shown to have a potential for activating the immune system, and may improve recovery from bacterial infections in skin lesions with added antibiotic medication in acute infections and without added antibiotic medication in less severe chronic skin infections. Propranolol may also have a preventive antibiotic effect.

Probiotic bacteria against multiresistant pathogens

Professor Iryna Sorokulova, Auburn University, Auburn, AL, USA

Extensive use of antibiotics in medicine and veterinary resulted in the emergence of antibiotic-resistant bacteria, which has become a serious problem worldwide. In previous years successful response to emerging resistance was discovering of new antibiotics. Today this approach has failed, because resistant strains are accumulated faster than new antibiotics are developed. Probiotic bacteria with pronounced activity against multiresistant pathogens could be valuable alternatives to antibiotics. We showed that Bacillus probiotic strain effectively inhibited antibiotic-resistant pathogens in vitro. Activity of this strain was caused by production of antibiotic with unique spectrum of activity and biosurfactants.

Clinical Application of Probiotics in Antibiotic Therapy: the Current and Future Use

Dr Thomas A. Tompkins, Research Director, Lallemand Health Solutions Inc, Montreal, Canada

This presentation will explore the background and our current knowledge of using probiotic microbes as an adjunctive or alternative therapy to antibiotic use. Clinical evidence will be presented on the value of probiotics in reducing side-effects of antibiotic therapy and the improved efficacy of probiotic + antibiotic co-therapy. We will discuss why live probiotic microbes can be effective even when given concomitantly with antibiotics to which they are sensitive. Issues surrounding the correct posology of probiotic co-therapy will be addressed. Finally, we will discuss future options in adjunctive probiotic therapy to reduce our reliance on antibiotics.

Oral Presentation Abstracts

CLINICAL EFFECT OF ANTIMICROBIAL PHOTODYNAMIC THERAPY WHEN USED WITH ROOT SURFACE DEBRIDEMENT FOR TREATMENT OF CHRONIC PERIODONTITIS- AUDIT OF TREATMENT RESULTS

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Background: Antimicrobial photodynamic therapy (aPDT) has a strong bactericidal effect. It was proven to inactivate and suppress virulence factors participating in the development of periodontal lesion. Application of aPDT has no known side effects and its action is localised and self-limiting. Repeated application is not associated with the rise of bacterial resistance. Improved clinical outcomes were reported when used in conjunction with mechanical root surface debridement.

Methods: Clinical effectiveness of the use of aPDT as an adjunctive treatment with mechanical root surface debridement was assessed in 25 cases treated in private clinical practice. All cases were diagnosed with chronic periodontitis and aPDT was used as part of first cycle of non-surgical periodontal treatment. Periodontal pocket depth (PPD) was used as primary outcome parameter. Changes in PPD for sites 4-6 and >=7 mm were calculated and compared with Cobb's data.

Results: In a mixed group of patients, which included both smokers and non-smokers, additional PPD reduction of 0.13 and 0.64 mm were observed in sites 4-6 and >=7mm deep respectively. Subgroup of non-smokers with averaged FMPS <20% demonstrated additional PPD reduction of 0.34 and 0.95mm in the same site categories.

Conclusions: Adjunctive use of aPDT improves clinical outcomes of non-surgical periodontal therapy with no known side effects associated with its application. Added benefit is greater in sites >=7mm where PPD reduction has improved by 29.6% when compared to mechanical debridement alone. Achieved clinical data support adjunctive use of aPDT as an alternative to antimicrobials for treatment of chronic periodontitis.

COMPUTATIONAL STUDIES OF ANTIMICROBIAL PEPTIDES

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Antimicrobial peptides (AMPs) are thought to kill bacteria by permeabilizing their lipid membranes. We recently used implicit membrane modeling to show that four AMPs bind more strongly to membrane pores than to the flat membrane, consistent with the idea that they stabilize them. In some cases the stronger binding to pores seems to arise from the imperfect amphipathicity of these peptides. One peptide exhibiting striking imperfect amphipathicity is protegrin. We have found that protegrin requires the positive curvature of toroidal pores to bind to neutral membranes. Models of protegrin oligomeric beta barrels have been constructed and evaluated energetically. The NCNC parallel topology was found to be most stable, in apparent disagreement with solid state NMR data. We have extended this approach to pores in anionic membranes and used it to obtain insights into the lipid selectivity of AMPs. Ideas emerging from these continuum studies are tested with explicit membrane simulations, some on the μs timescale. Such simulations showed that incomplete barrels (arcs) of protegrin were also stable for at least 2 μs.

MOLECULAR AND GENETIC CHARACTERISATION OF ANTIBIOTIC RESISTANCE GENES AND MOBILE GENETIC ELEMENTS IN KLEBSIELLA PNEUMONIAE STRAINS ISOLATED FROM TRIPOLI & BENGHAZI, LIBYA

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Abstract: Klebsiella pneumoniae is the most frequently isolated species from ICU wards, hospital environment and medical devices in Tripoli and Benghazi. As the current rate of antimicrobial resistance among K. pneumoniae in Libya is unknown, this study was undertaken.

Methods & Materials: A total of 80 clinical and environmental isolates of Klebsiella pneumoniae were collected from Tripoli and Benghazi. PCR was used to detect the occurrence of class 1 integrons, transposons and CTX-M type Extended Spectrum β-lactamases (ESBLs). Transconjugation experiments were performed on a subset of K. pneumoniae using E. coli J53 as a recipient, S1 genomic digests followed by probing with CTX-M-15 and ISEcp1. Random amplified polymorphic DNA (R APD) typing was conducted to the study the clonal relatedness between K. pneumoniae strains. Pulsed Field Gel Electrophoresis (PFGE) was also used to type the strains Multi-locus Sequence Typing (MLST) was also performed to study which sequence types were the most common hosts of blaCTX-M-15.

Results: Sequencing of PCR products showed the occurrence of CTX-M-15 gene associated with its insertion sequence ISEcp1. Transconjugation experiments showed that a movement of blaCTX-M-15 from parents to transconjugants had occurred. Probing experiments showed that CTX-M-15 and ISEcp1 genes were detected on plasmids of different sizes; 50, 75, 100, 150, 275, 300 and 425 kb in both parents and transconjugants. RAPD demonstrated 6 main clusters contained a collection of different strains similar to each other and some of them are clonal.after having performed further analysis. PFGE showed that some of the MDR isolates are clonal and were collected from patients and ICUs environment. MLST results showed that STs 15, 29, 70, 110, and 111 were detected carrying CTX-M-15 genes.

Conclusions: These results show the spread of different sequence types of endemic multi-drug resistant strains of K. pneumoniae in Tripoli and Benghazi. blaCTX-M-15 encoding gene was detected chromosomally and plasmid mediated in clinical and environmental strains.

THE SEA STAR ECHINASTER SEPOSITUS: A NEW SOURCE FOR BIOACTIVE COMPOUNDS

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Marine invertebrates are commonly considered as an interesting source of new bioactive molecules, useful for applications in bio-medical field. In many case, these molecules are important components of the innate immune system of invertebrates. Sponges, coelenterates, tunicates and echinoderms are some examples of natural products producers. Some compounds isolated from marine invertebrates, such as from Echinoidea, are antimicrobial peptides active against Gram positive and Gram negative bacteria and yeasts. These compounds show a broad antimicrobial spectrum including also human pathogens. In this work mucus, cell free coelomic fluid and coelomocyte lysate of the red sea star Echinaster sepositus were investigated in order to evidence the presence of bioactive compounds.

Adult specimens of E. sepositus were collected in a coastal area of the Northern Ionian Sea (Porto Cesareo, Lecce, Italy) and after collection, mucus, cell free coelomic fluid and coelomocytes were pooled to test, hemolytic, hemagglutination and lysozyme-like acitivities The lysozyme-like activity was estimated by using the test of Micrococcus luteus cell wall lysis, conducted on Petri dish and expressed as diameter of lysis produced. Human red blood cells were employed for the spectrophotometrically evaluation of the hemolytic activity and for the hemoagglutination microtitration assay. Furthermore, to verify the non-toxicity of the samples, the classical MTT test and the specific Microtox® test against Vibrio fischeri were performed. A noticeably lysozyme-like activity was found in all the studied compartments. A high ability of coelomocyte lysate as well as mucus to lyse human red blood cells was recorded. Moreover, mucus, cell free coelomic fluid and coelomocyte lysate showed a strong ability to agglutinate human erythrocytes. Finally by the toxicity assays we evidenced that samples resulted non toxic. Such a result is noteworthy for future applications in the pharmaceutical field that requires non-toxic compounds for the development of new drugs. All these data represent an important clue to better understand the defence mechanisms of the here investigated asteroid, but they are also interesting for the potential biotechnological implications. Indeed, if further investigations will support these data, they may suggest the use of Echinaster sepositus as a source of bioactive compounds.

THE LIPIDIC EXTRACT OF THE SEAWEED CYSTOSEIRA BARBATA (STACKHOUSE) C. AGARD: A POTENTIAL SOURCE OF BIOACTIVE COMPOUNDS

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Seaweeds represent one of the most important marine renewable resources and produce structurally novel secondary metabolites with antibacterial, antialgal, antifungal, antitumoral and antioxidant properties. Therefore, natural products from marine algae appear as a promising alternative valuable source of new compounds for drug development. In particular the application of seaweeds antioxidants in foods, food supplements, nutraceuticals and medicine is recently considered from the perspective of benefits to human health. Moreover seaweeds often produce salt-stable antimicrobial compounds useful in the control of fish or shellfish pathogens in aquaculture where the high-salt conditions might reduce antibiotic efficacy. In this investigation the antimicrobial and antioxidant activity of Cystoseira barbata lipidic extract was assayed The seaweed was collected in the Mar Piccolo of Taranto (Northern Ionian Sea, Italy), washed, dried and powdered. The powdered material was subjected to Soxhlet extraction using choloroform/methanol (2:1 at 55-60 °C for 24 h). Five milligrams of extract were dissolved in ethanol and assayed for antimicrobial activity using the Kirby Bauer method (1966). The antioxidant activity of the extract was measured using two in vitro assays: the TEAC (Trolox Equivalent Antioxidant Capacity) assay based on a single electron transfer (SET) reaction, using ABTS [2-2'-azino-bis (3-ethylbenz-thazoline-6-sulfonic acid)] as chromogen and the ORAC (oxygen radical absorbance capacity) based on a hydrogen transfer mechanism (HAT). The antioxidant activity of the extract measured by TEAC and ORAC assays were respectively equivalent to 225,19±10,99 and 800,67±180,24 μmol Trolox Equivalent/g extract. These antioxidant values are comparable with those reported in the literature for various high antioxidant fruit and spice extracts. Cystoseyra barbata lipidic extract showed a bacteriostatic activity against Pseudomonas aerugionsa as well as all the Vibrio species utilized (Vibrio alginolyticus, Vibrio vulnificus, Vibrio cholerae non O-1). These results are noteworthy considering the resistance against antibiotics developed by bacteria and the need to control fish and shellfish diseases due to vibriosis. The antioxidant activity was also of interest taking into account the potential for protective effects of seaweed against lipid oxidation in food and oxidative stress in target tissue and LDL in vivo