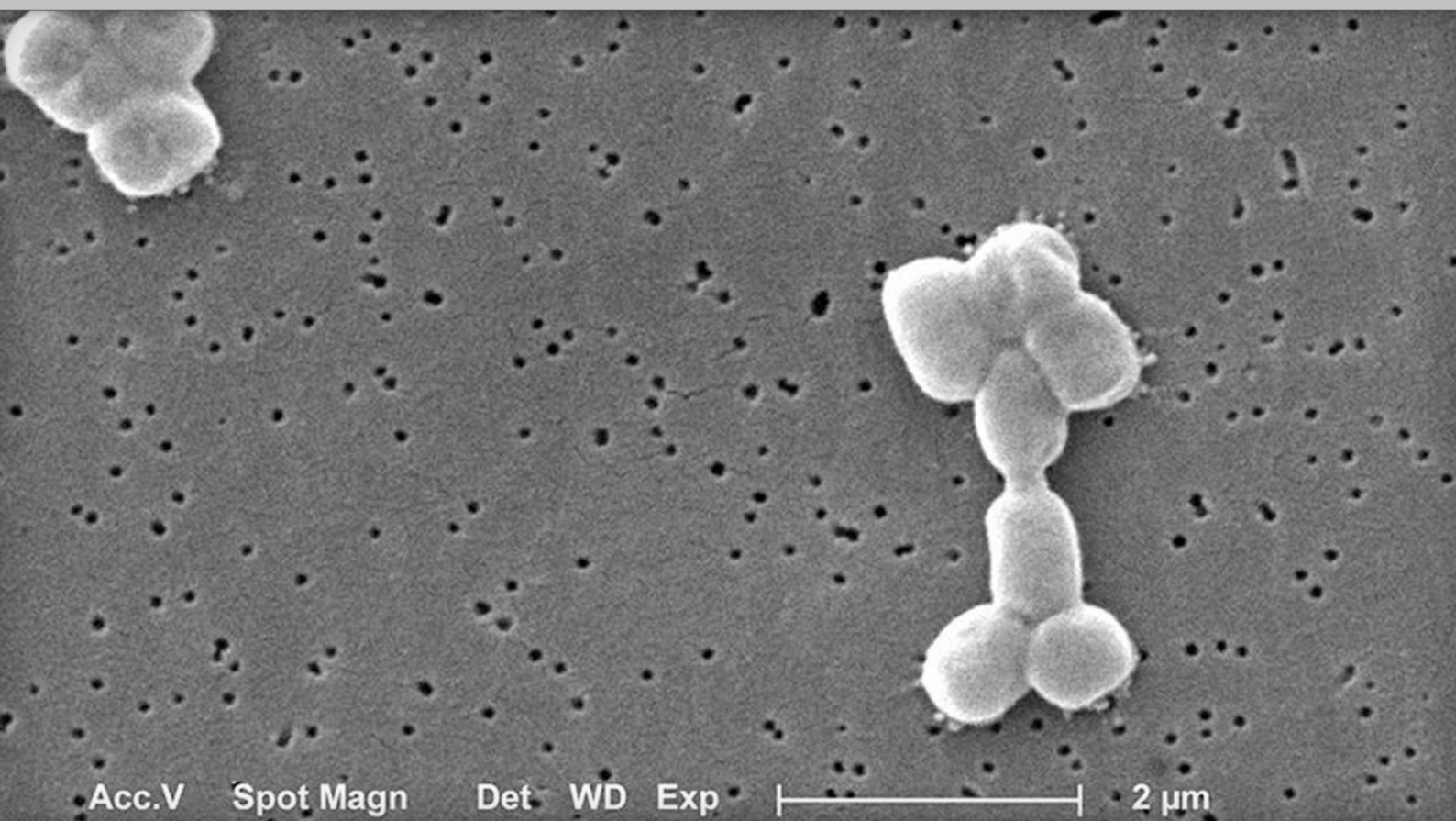


ANTIBIOTIC RESISTANCE AND ANTIBIOTIC ALTERNATIVES

LOOKING TOWARDS THE FUTURE

ABSTRACTS



3rd - 5th November 2015
London, UK

EuroSciCon 

This international interdisciplinary event is an open forum for discussion of the rise in antibiotics resistance and the potential utility of various antibiotic alternatives in treating human and animal disease. Using a multi-professional and inter-specialty approach this event promises plenty of opportunity for discussion and debate set in an informal atmosphere.

This event has [CPD accreditation](#)

This abstract book will be finalised two weeks before the event

www.regonline.co.uk/antibio2015

Hashtag: #Antibio2015

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Invited Speakers Abstracts

Nanoparticles and the control of infections.

Professor Robert Allaker, Queen Mary University Of London, London, UK

Nanoparticles, novel in size, shape and surface chemistry may offer functional properties to a range of health care applications. Possible uses as constituents of prosthetic device coatings, topically-applied agents and within biomaterials are being explored. In particular, the potential to control infections through their use as antimicrobial and osteoconductive implant coating materials. Whereby, the potential of nanoparticles to control the formation of biofilms, as a function of their biocidal, anti-adhesive and delivery capabilities has been assessed. Exploitation of the toxic properties of nanoparticles to microorganisms, in particular copper, zinc and their oxides has demonstrated key candidates for future use.

Microcins as antibiotic alternatives: Structural basis for hijacking outer membrane siderophore receptors by antimicrobial peptides

Dr Konstantinos Beis, Senior Lecturer, Imperial College London, Membrane Protein Lab RCaH, London, UK

Microcins are gene-encoded antibacterial peptides produced by Enterobacteriaceae. The MccJ25 is a posttranslationally modified peptide antibiotic that adopts a lasso structure and exerts potent antibacterial activity against closely related species. The uptake of MccJ25 into bacterial cells is facilitated by the outer membrane siderophore receptor FhuA and inner membrane protein SbmA. We have obtained the crystal structure of the FhuA in the presence of MccJ25. FhuA is 22-stranded antiparallel beta-barrel, which is obstructed by a plug domain. An essential hydrogen-bond between the FhuA plug domain and MccJ25 induces a transport event. The structure provides useful information on understanding how microcins hijack outer membrane receptors.

Repurposing non-steroidal anti-inflammatory drugs (NSAIDs) in the fight against tuberculosis (TB)

Dr. Sanjib Bhakta, Institute of Structural and Molecular Biology, Birkbeck, University of London and UCL, London, United Kingdom

Development of an effective drug treatment with novel mechanisms of action is urgent to tackle antibiotic resistance in tuberculosis (TB). Novel chemical entities require at least a decade to be commercially available therefore repurposing over-the-counter drugs offer a solution to circumvent the investment of time and other resources. Certain common non-steroidal anti-inflammatory drugs (NSAIDs) have proven to be selectively bactericidal against replicating, non-replicating and multi-drug-resistant clinical isolates of *M. tuberculosis*. Our primary focus is to repurpose common NSAIDs and investigate their novel mechanisms of action in *M. tuberculosis* to help design more potent inhibitors in the future.

Targeting bacterial exotoxins with liposomes

Professor Annette Draeger, Abteilung Zellbiologie, Institut für Anatomie, Universität Bern, Bern, Switzerland

Gram-positive bacterial pathogens that secrete cytotoxic pore-forming toxins, such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, cause a substantial burden of disease. We have engineered artificial liposomes, tailored to effectively compete with host cells for toxin binding. Artificial liposomes act as decoy targets to sequester bacterial toxins that are produced during active infection in vivo. Their administration within 10h after infection rescues mice from septicemia caused by *S. aureus* and *S. pneumoniae*, whereas untreated mice die within 24–33h. Tailored liposomes are not bactericidal, therefore do not promote antibiotic resistance. They could be used alone or in conjunction with antibiotics.

Should Homeopathy be considered as part of a treatment strategy to replace antibiotics in otitis media with effusion in children? A review of the evidence

Mrs Alison Fixsen, BA (Hons), MA, MBSH, RSHom, PGCHE, DipNP. Senior Lecturer and Deputy Disability Tutor Faculty of Science and Technology University of Westminster, London, UK

Otitis media with effusion (OME) or 'glue ear' is the most common cause of pediatric hearing loss, and a drain on global health care resources. Socio-economic and environmental factors play a significant part in severity and impact of OME on cognitive and developmental outcomes. Current conventional treatment strategy for OME is beset with concerns about excessive or inappropriate antibiotic use, while homeopathy trials show positive results. In this presentation I consider the social and cultural implications of OME, its impact on high-risk individuals and communities, and propose an integrative approach to OME, including the use of global small-scale homeopathy trials.

Management of bite wounds in children and adults – an analysis of over 5.000 cases at a Level I Trauma centre

Dr Florian M. Kovar, Univ. Lektor-Dr. Florian M. Kovar, Attending for Trauma Surgery, Medizinische Universität Wien, Universitätsklinik für Unfallchirurgie, AKH Wien, Austria

Bite wounds account for 5% of the total traumatic wounds evaluated in the ED (emergency department) and approximately 1% of all ED visits. Early estimation of infection risk, adequate antibiotic therapy and if indicated surgical treatment, are cornerstones of successful cure of bite wounds. 5.248 consecutive trauma patients were collected. Total infection rates within 24 hours to antibiotic administration was 29.3%, compared to 65.0% <48 hours, and 81.1% <72 hours.

Avian antibodies (IgY) to fight antibiotic resistance

Dr Hans Kollberg, Uppsala University, Sweden, UK

Avian antibodies; replacement to antibiotics. Diminish antibiotic resistant microbes. Can be used for immunotherapy of infectious diseases. Microorganisms have never become resistant towards antibodies.

Eggs: normal diet; no risk for advert events.

IgY: peroral immunotherapy of infections in mouth, throat, stomach, intestine, airways, skin.

IgY: cheap, can be scaled up to big quantities; lenient for hens (no bleeding needed).

Convincing results from studies *in vitro*, animals, humans. Anti-pseudomonas IgY to CF-patients has been running >19 years. Phase II: statistically significant advantage for IgY patients versus those without therapy. Phase III study still running. Latest reports given at the meeting.

The resurgence of penicillin-susceptible *Staphylococcus aureus* from infectious keratitis

Professor Regis P. Kowalski, MS, M(ASCP), Research Professor of Ophthalmology, Executive Director of The Charles T. Campbell Ophthalmic Microbiology Laboratory, University of Pittsburgh, Pittsburgh, PA, US

The MICs of *Staphylococcus aureus* isolated from cornea samples to penicillin has decreased over the last 20 years indicating that topical penicillin may be returning as an effective antibiotic for the treatment of keratitis. High antibiotic concentrations are achieved in the cornea due to topical therapy. Systemic therapy is never used to treat corneal infections. Penicillin may be a potent and cost-effective therapy for MSSA keratitis.

Effects of feeding waste milk to dairy calves: bacterial antibiotic resistance and respiratory and intestinal microbiota population

Georgina Maynou, Institute of Agrifood Research and Technology (IRTA), Catalonia, Spain

The purpose of this study was to assess the effect of feeding calves pasteurized waste milk (pWM) on bacterial antimicrobial resistance development and composition of bacterial communities from respiratory and intestinal tract. The results obtained from antibiotic susceptibility tests indicated that feeding calves pWM triggered the presence of β -lactam resistant *E.coli* in the gut of dairy calves. This effect disappeared after weaning once antibiotic residues from milk ceased. Gut and respiratory tract microbiota phylogenetic analysis showed a decrease in Bacteroidia class from gut microbiota and Bacilli class from nasal microbiota of calves fed pWM compared with those fed milk replacer.

Boosting your immune system using fragments of innate immunity proteins

Dr Jens Madsen, Lecturer in Child Health PhD, Sir Henry Wellcome Laboratories, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK

The innate immune system has developed over time in parallel with the human development. Many of the proteins in innate immunity are directed against conserved structures on micro-organisms that are vital for the survival of the organism. This talk will discuss the potential of using recombinant innate immunity proteins as alternatives to antibiotics, especially focusing on surfactant protein D (SP-D), which is expressed on localised on mucosal surfaces.

Does the presence of antimicrobial substances increase the prevalence of community acquired MRSA?

Dr Stephen Mortlock, Q² Solutions, University of Surrey, London, UK

Staphylococcus aureus is a major cause of hospital-acquired infections, leading to high morbidity and mortality worldwide. The incidence continues to increase at an alarming rate, particularly in less developed countries, where there may be widespread misuse of antibiotics. Once thought to be confined to hospitals, strains of MRSA are now known to occur in the community (CA-MRSA) and can be attributed to previous antimicrobial therapy, or previous hospitalisation. A large proportion of the CA-MRSA patients attending the SKMC hospital in Lahore, Pakistan demonstrated the presence of antimicrobial substances, and it had been theorised that this contributed to the presence of the MRSA.

Targeting host CDC42 as a therapeutic strategy for limiting invasive bacterial infection

Professor Susan McDowell, PhD, Director, Biotechnology Program, Ball State University Muncie, Indiana, USA

The global emergence of invasive infection by *Staphylococcus aureus* and by *Streptococcus pyogenes* is associated with life-threatening disease recalcitrant to current antibiotic therapies. We have identified a series of molecules that limit invasive infection by acting at the level of the host: through inhibition of the host protein CDC42. Each of the small molecule inhibitors shows selectivity for the small guanosine triphosphatase, inhibits invasiveness through non-cytotoxic/non-bactericidal mechanisms, and acts on key host cellular pathways exploited by *S. aureus* and by *S. pyogenes*. Further development of these molecules has the potential to provide adjunctive therapeutic approaches to mitigate invasive infection.

A daily probiotic as an alternative treatment strategy for recurrent diverticulitis

Dr John Nichols, SW Thames Faculty of the Royal College of General Practitioners, University of Surrey, Guildford, UK

There is anecdotal evidence that consumption of certain probiotic strains may control recurrent diverticulitis so well that antibiotics are never needed. Several small European trials have shown benefits from various treatment combinations involving probiotics (mainly in combination with a prebiotic or mesalazine) but one USA trial failed to show any advantage of giving the probiotic *Bifidobacterium infantis*. Our pilot study of a daily dose of *Lactobacillus casei* Shirota (Yakult) showed benefits for most participants including a reduced need for antibiotics.

Anti-infectives from marine resources - A better alternative to antibiotics to treat nosocomial and opportunistic pathogens

Professor S Karutha Pandian, Professor & Head, Department of Biotechnology & Dean: Faculty of Science & Member of Vice-chancellor, Officiating Committee, Alagappa University, Science Campus, India

The unprecedented deployment of antibiotics in the treatment scenario leads to the genesis of Multidrug resistance Pathogens (MDR). Most of the nosocomial and opportunistic pathogens are MDR pathogens which pose a severe challenge in the current treatment strategies. One among the novel ways to treat the microbial pathogenesis is to target cell to cell communication pathways of the MDR pathogens in the form of Anti-infectives through Quorum sensing mediated inhibition of virulence factors. Exploration of newer anti-infectives from natural resources with special reference to bacteria through culture dependent and independent techniques will act as a novel alternative to the present day antibiotics

The future of anti-virulence therapy

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

How could we imagine the antibiotic of future, which additional characteristics should it have? Starting from a good selectivity index, other important properties will be needed: the ability to hit pathogens without killing beneficial microbiota; a low selectivity pressure to promote the rise in antibiotic-resistance strains; the property to tackle natural form of resistance like the biofilms; the capacity to eliminate microbial cells metabolically inert. The possibility of using novel agents that target virulence mechanisms and biofilm formation offers potential for new therapeutic strategies for the treatment of chronic bacterial infections and could significantly impact on overcoming the problem of antibiotic resistance.

The value of antibiotics in an era of escalating bacterial resistance

Dr Glenn Tillotson, Transcrip Partners, Downingtown, PA, USA

Antibiotic resistance among community and hospital pathogens is a growing health issue. The usual outcomes of resistant organisms include poor clinical efficacy, longer stays in hospital or ICUs, and mortality. The significant financial and productivity impact due to these growing infections is not recognised. In my presentation, I will present the new perspective on the overall impact of antibiotic resistance, the current resistance epidemiology, the antibiotic pipeline from 2015 onwards and how we all must shift our perception of the real value of antibiotics or we will face a very bleak future as many daily medical procedures will become impossible.

Bacteriophages controlling Extensively Drug resistant E. coli sequence types

Dr Mark A. Toleman, Senior Lecturer, Department of Infection and Immunity, Cardiff University, London, UK
Antibiotic resistance is generally very closely linked with only a few strains of the many strains found within an individual bacterial species yet the underlying reasons behind this are poorly understood. Recently a powerful resistance mechanism emerged in South Asia (The New Delhi Metallo- β -lactamase) which is able to make bacteria extensively drug resistant and in some cases pan-resistant to current antibiotic therapy. Here we show that environmental bacteriophage control the prevalence of individual strains of E. coli carrying this resistance mechanism. This helps us both understand the close linkage between antibiotic resistance and individual strains and also suggests that phage could be usefully employed to manipulate strains thus reducing antibiotic resistance.

Emerging resistance towards antibiotics – a burning problem

Dr Prakash U. Tahlilani, Clinical Researcher, Prime Ever Ayurvedic Research Laboratories, Navsari, Gujarat, India
WHO's 2014 report on global surveillance of anti-microbial resistance revealed that antibiotic resistance is no longer a prediction for the future; it is happening right now, across the world and has potential to adversely affect the ability to treat common infections. Without urgent, co-ordinated action, the world is heading towards a post antibiotic era which may be similar to pre antibiotic era killing many people with common infections. As an urgent step to cope up with the problem of antibiotic resistance, we may think of developing new herbal combination remedies.

Antibiotic resistance: on the importance of antibiotic efflux pumps

Dr Hendrik W. Van Veen, University of Cambridge, Cambridge, UK
Antibiotic resistance associated with bacterial infections is a growing problem worldwide. Research aimed at a deeper understanding of the mechanisms that bacteria employ to overcome toxicity of antibiotics helps to understand how to combat this public health issue. In this presentation I will review basic mechanisms of drug resistance, and emphasise the important roles of drug efflux pumps in both the intrinsic and acquired resistance to antimicrobial agents. Structural and biochemical studies have greatly advanced our insights into the working mechanisms of drug pumps. The implication of these studies for improved therapy will be discussed.

Day 1:

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

BACTERIOSTATIC AND BACTERICIDAL ACTIVITIES OF VANCOMYCIN AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS. SYNERGISTIC POTENTIAL COMBINATION WITH OTHER ANTIMICROBIAL AGENTS BY TIME-KILL CURVE METHODS

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common pathogens that cause serious infections in patients. Despite the existence of new antimicrobial agents, vancomycin (VAN) remains the standard therapy for treating infections caused by these strains. Antimicrobial susceptibility testing is an important challenge for the laboratory. At present, the most frequently used methods are: the Minimum Inhibitory Concentration (MIC), the Minimum Bactericidal Concentration (MBC) and time-kill studies (TKS) or killing curves (KC). The tolerance is defined taking into account two criteria: MBC/MIC ratio and TKS.

The aims of this study were: a) to detect VAN bacteriostatic and bactericidal activities; b) to distinguish tolerant strains according to different criteria; c) to study VAN synergical activity with ciprofloxacin(CIP), gentamicin(GEN), rifampin(RFA) and imipenem(IMI) against VAN-tolerant MRSA isolates by time-kill studies (TKS).

One hundred and twelve MRSA of clinical significance were studied for a period of two years. The isolates were unique and consecutive and were obtained from patients attending the Dr. José María Cullen Hospital, a teaching Hospital in Argentina.

VAN MIC and MBC studies were performed by broth macrodilution (CLSI). TKS were used with isolates showing VAN MBC/MIC ratio ≥ 8 , counting survivors at 0, 3, 6 and 24 h of incubation at 35° C. TKS were also used to study synergism effect with CIP, GEN, RFA and IMI against tolerant MRSA isolates (NCCLS,1999).

The VAN MIC_{50/90} were 0,5 and 1 µg/mL, while the MBC_{50/90} were 1 and 8 µg/mL, respectively.

Considering MBC/MIC ratio VAN bactericidal activity was 65,2% and 12 strains (10,7%) were tolerant.

TKS were performed against 39 isolates that showed VAN MBC/MIC ≥ 8 . VAN showed bactericidal activity for 96,4% strains tested, while 4 isolates resulted tolerant (3,6%). Interestingly one of them was not tolerant according to MBC/MIC ratio.

The MIC of the other studied antimicrobial agents against VAN tolerant MRSA were categorized as susceptible, including imipenem (IMI). When TKS were performed, all of them were bactericidal after 24 hours of incubation. All antimicrobial combinations were synergistics, but VAN with GEN, RFA and/or IMI combinations achieved synergistic activity at 6 hours of incubation while the VAN with CIP combination was synergistic at 24 hours against 3 tolerant MRSA. None of the antimicrobial combinations studied were antagonistic.

It is concluded that: a) VAN may continue to be used in this teaching hospital; b) to study antimicrobial bactericidal activity is recommended to use TKS because there were discrepancies in the detection of tolerant strains according to different criteria; c) antimicrobial combinations to treat VAN tolerant MRSA are highly effective due to its strong synergistic response.

DIRECT MODIFICATION OF PHOTSENSITIVE NON-ANTIBIOTICS BY EXPOSURE TO UV LASER RADIATION AND THEIR INTERACTION WITH TARGET SURFACES: NEW PROCESS FOR DRUG DISCOVERY AND DELIVERY IN VIEW OF SPACE MISSION APPLICATIONS

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Since time spent in space by humans increased with the advancement of explorations and multiple drug resistance evolved, the interest in developing new methods to treat human patients and to decontaminate spacecraft's surfaces in case of infections significantly increased.

On one hand, human immune responses are weakened in space, therefore the body is more susceptible to infections; on the other, microbes can survive, proliferate and exhibit enhanced virulence and resistance to environmental stresses in micro- and hypergravity conditions. For this reason, humans and spacecraft components may need special treatments during space missions.

A solution designed to overcome these challenges related to microbial infections onboard consists in the use of UV laser beams in interaction with medicine containing solutions to induce structural changes at their molecular level and to obtain new photoproducts with possible antimicrobial effects.

Impregnation of target surfaces with such solutions is relevant for developing new tools in targeted drug delivery. The main scientific objective is to investigate the interaction of unexposed and exposed to UV laser radiation medicine solutions with surfaces.

The wettability of surfaces in terms of contact angles were determined under terrestrial gravity conditions at the liquid-solid-air interface of a sessile drop containing medicine solutions. The results showed that drug solutions have better than water wetting properties on the target surfaces; the contact angles of drug containing droplets exhibited values less than 90 degrees which indicates favorable surface wettability.

Within ESA "Spin Your Thesis!" 2015 programme, the wettability of target surfaces by medicine solutions is studied under hypergravity conditions.

IS IT POSSIBLE TO CONTROL ANTIBIOTIC RESISTANCE IN ECUADOR? 15 YEARS OF VIGILANCE

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Introduction.

When searching for data on antibiotic resistance in Latin America, there are few studies published, the little data out there is from but a few countries. When comparing figures from punctual, national and regional data, the data is contradictorily; often punctual studies present much higher rates of resistance than national data. This is widely attributed to underreporting and a prevalent attitude in governments to maintain the illusion of total control. Many studies from Latin America do not have sufficient depth, with inadequate numbers of cases, making many studies, of limited scientific value. Surveillance of antimicrobial resistance allows clinicians to monitoring crucial changes in populations of microorganisms, allows for the early detection of resistant strains important for public health, and facilitates rapid reporting and investigation of outbreaks. In the hospital environment, monitoring is essential for an informed treatment plan; it guides policy, through recommendations based on resistance patterns, enables effective empirical therapy and assesses the impact of interventions to contain resistance.

Objective.

The aims of this study are: 1. To evaluate the evolution, over 15 years, of antibiotic resistance patterns in the Vozandes hospital, in Quito. 2. To investigate the cause of significant diversity in resistance rates between the Vozandes hospital and other participating hospitals in the national vigilance network.

Material and Methods.

For fifteen years (2000-2014) patterns of antibiotic resistance have been monitored in a continuing surveillance program, in the Vozandes hospital, and recorded in database software developed for the management and analysis of microbiology laboratories (WHONET). All data from the Vozandes hospital were obtained during routine clinical care. Data from vigilance network hospitals in Ecuador, were reported directly to the PAHO and published in annual reports (2000 to 2009). From 2010 to 2013 data was reported to the WHO, Global Report on Surveillance and from 2014, data has been reported to the National reference laboratory.

Results.

All years between 2000 and 2014, there are statistically significant differences between the hospital Vozandes and national data. Data shown below (2014) are representative of all years. E. coli in Urinary Tract Infections (n=1914) Hospital Vozandes resistance to ceftriaxone is 20.5%, while the national level is 37% (n=3317) p

Conclusion.

The microbiology laboratory is a fundamental tool for health professionals and public health authorities to determine the source of an outbreak and which antimicrobials represent effective treatment options. This requires well trained, motivated, laboratory professionals, equipment, materials and protocols to ensure effective communication with clinicians and public health authorities. If integrated correctly, a microbiology laboratory can prevent resistance from occurring, halt the spread and save substantial economic burden associated with prolonged costly treatment. For 15 year clinical data was collected and inputted into a database, this has afforded a unique perspective of resistance in Ecuador, unmatched in its resolution, especially when examined next to national data. When comparing the Vozandes hospital, with 15 years of detailed data confirming the continuing low rates of resistance, to national data, there is a stark difference. The resistance rates in the Vozandes hospital are less than half of that found at a national level, for most of the strains; however, when considering that national data are underreported, the gap becomes greater. There are many possible factors to consider when looking for an explanation. The Vozandes is a small (76 bed) private hospital that caters for the economic middle/upper class, whereas the majority of the vigilance network are larger (>200 beds) public hospitals that cater mainly to the economic lower class, therefore, it is necessary to make allowances for a disparity in budgets, bed turnover rates and associated medical socioeconomic factors. Despite these differences in the hospitals, it is unlikely that they are solely responsible for such a significant disparity. A potential factor that affects public hospitals is that disputes are commonplace between the hospital administration and staff, over the procurement and need of materials; a dispute that often leaves hospitals short of sufficient levels of the essentials. Hygiene, especially the standard of which cleaning within hospitals is conducted, is of greatly varying standards. In the Vozandes hospital, hygiene protocols are updated as and when new advice is given, the importance pressed upon staff and adherence to cleaning routines is enforced. In contrast, public hospital staff, backed by unions, are often reluctant to adopt new routines and hospital management find that they are hindered in taking action to enforce, good professional practice. In the absence of compulsory reporting to a vigilance network, but a few hospitals voluntarily submit reports and that that is reported is dependent on professionals submitting work through the chain. If at any point the chain is broken, the data is lost. Additionally, there is no parallel organization to accompany the vigilance network, to monitor antimicrobial consumption; the quality of data recorded in individual case histories submitted to the vigilance network being the only data available. Knowing antimicrobial consumption and integrating it into resistance data, would provide crucial data to inform the necessary political commitment required to achieve successful campaigns against antibiotic resistance.

Day 2:

Oral Presentation Abstracts

PHARMACOKINETICS OF SULPHADIMIDINE IN NON-STARVED AND STARVED FEMALE TURKEYS

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Sulphadimidine, a systemic sulphonamide, has maintained an active place in the armamentary of antimicrobial drugs used in the treatment of infectious diseases in turkeys. Recently it has been discovered that starved male turkeys eliminate sulphadimidine slower than non-starved male turkeys, suggesting the possibility of prolonged effect and, invariably reduced resistance in the starved turkeys. In view of this, comparative pharmacokinetic study of sulphadimidine was carried out in non-starved and starved female turkeys of 12weeks old weighing 1.37 ± 0.03 and divided into two groups of ten each. One group was administered a single intramuscular dose of 100mg/kg body weight of sulphadimidine. The other group was kept off-feed for 48hours before administration of 100mg/kg body weight intramuscular sulphadimidine. The result showed that the concentration maximum ($C_{max}=91.91\pm 6.58$ $\mu\text{g/ml}$), elimination rate constant ($\beta=0.08\pm 0.01$ hr^{-1}), elimination half-life ($t_{1/2\beta}=9.99\pm 1.31$ hr), body clearance ($Cl_b=0.10\pm 0.01$ L/kg/hr), mean residence time ($MRT=14.40\pm 1.80$ hr), area under curve from zero to 96hours ($AUC_{0-96}=1.57\pm 0.15$ mg/L/hr), area under curve from zero to infinity ($AUC_{0-\infty}=1.58\pm 0.15$ mg/L/hr), area under moment curve ($AUMC=23.62\pm 3.96$ mg/L), and volume of distribution of central compartment ($V_c=1.62\pm 0.11$ L/kg) were significantly lower ($p<0.05$) in non-starved in comparison with the ($C_{max}=128.04\pm 6.97$ $\mu\text{g/ml}$), ($\beta=0.053\pm 0.01$ hr^{-1}), ($t_{1/2\beta}=14.39\pm 1.52$ hr), ($Cl_b=0.06\pm 0.01$ L/kg/hr), ($MRT=20.41\pm 2.08$ hr), ($AUC_{0-96}=2.64\pm 0.31$ mg/L/kg), ($AUC_{0-\infty}=2.72\pm 0.34$ mg/L/kg), ($AUMC=57.89\pm 1.18$ mg/L), and ($V_c=1.07\pm 0.08$ L/kg) of the starved turkey. In conclusion intramuscular 100mg/kg body weight of sulphadimidine in starved female turkeys may likely prolong its antimicrobial effect and residue resistance.

ALTERNATIVE TO ANTIBIOTICS - PHOTODYNAMIC ERADICATION OF PATHOGENIC BACTERIA

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Unsuitable, uncontrolled and excessive use of antibiotics has caused drug resistance of pathogenic bacteria. Photodynamic antimicrobial chemotherapy can become a good alternative to antibiotic treatment of bacterial infections. This technique is based on the use of dyes called photosensitizers, which can be activated by visible external indoor or outdoor illumination or by chemiluminescent and sonoluminescent light. Photosensitizers have no or very low toxicity in the dark. Furthermore, no bacterial resistance to photosensitizers has been reported to date.

In the present study, the antibacterial activity of the immobilized photosensitizers Rose Bengal as a sodium salt or in lactone form, methylene blue and hematoporphyrin was tested against Gram-positive *S. aureus* and *S. epidermidis*, and Gram-negative *E. coli* and *P. aeruginosa* cells. Immobilization was performed either by dissolution of photosensitizers and polymers in chloroform with further evaporation of the organic solvent and the formation of thin polymeric films, or by dissolution of the photosensitizers in the melted polymers to obtain polymeric rods or beads. All immobilized photosensitizers showed very high antibacterial activity against Gram-positive and moderate activity against Gram-negative bacteria. Free Rose Bengal when applied at sub-MIC concentrations and combined with kanamycin A, ampicillin or methicillin, increased the sensitivity of *S. aureus* to these antibiotics.

ARTILYSIN®S - ANTIMICROBIAL DESIGNER ENZYMES FOR TARGETED ELIMINATION OF BACTERIAL PATHOGENS

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Growing antibiotic resistance is of major concern worldwide. Broad use, or misuse of antibiotics is resulting in increasing levels of antibiotics present in the environment, leading to a growing antibiotic resistance. As it is getting clear that also low concentrations of antibiotics in the SUB-MIC range can induce antibiotic resistance, biodegradable antimicrobial solutions are becoming more and more attractive. Furthermore, there is a clear need for effective antimicrobial alternatives, which ideally show a targeted mode of action, - especially addressing *Pseudomonas* and other Gram-negative bacteria, leaving the body's natural microbiome intact. Artilysins constitute a novel class of efficient enzyme-based antibacterials with a new mode of action: Artilysins 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. Artilysin® Art-175 is highly effective against *P. aeruginosa*, a Gram-negative pathogen well known for being highly resistant to antibiotics and being responsible for re-occurring infections. Art-175 passes the outer membrane and kills *P. aeruginosa*, including multidrug-resistant strains, in a rapid manner: 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*, with a MIC₉₀ of 10 µg/ml independent of the strains being highly resistant to antibiotics. Resistance development against Art-175 was not observed within 20 experimental cycles on all strains investigated, whereas resistance development against a ciprofloxacin control occurred already within 7 cycles of the MIC experiments. As Artilysins do not require an active bacterial metabolism for its antibacterial activity, they show a superior bactericidal effect against persisters of *P. aeruginosa* and other bacterial species. Systemic infections by *P. aeruginosa* were successfully treated with Art-175 in a mouse model. Preclinical data underline the broad applicability of Artilysins to combat bacterial infections. In summary, Artilysins are proteins and thus fully biodegradable that are using a novel antibacterial mode of action for targeted elimination of infections caused by difficult to treat antibiotic resistant and/or persistent bacteria like *P. aeruginosa* that favour the microbiome.

Day 3:

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

BACILLUS BACTERIA AS ANTIMICROBIALS

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Bacteria of the *Bacillus* genus are ubiquitous in nature as a normal component of soil, water, air, food and gut microbiota. Bacilli are known for their high metabolic activity, particularly for their antagonistic activity against pathogens. More than 700 different antimicrobials were isolated from *Bacillus* bacteria. The antimicrobial activity of these bacteria can be also associated with the production of lytic enzymes. We isolated and identified a *Bacillus subtilis* strain with a wide spectrum of activity against pathogens, including antibiotic-resistant bacteria. The activity of this strain was shown in vitro, in animal models and in clinical trials. *Bacillus* bacteria can be successfully used as antimicrobials in human and veterinary medicine.

DYNAMIC TIME-LAPSE MICROSCOPIC STUDIES EMPLOYING MICROFLUIDIC-BASED TECHNOLOGY REVEAL THE KILLING KINETICS AND MECHANISMS OF SYNTHETIC PEPTIDES IN DIFFERENT PATHOGENIC BACTERIA

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Conventional *in vitro* antimicrobial susceptibility testing carried out on agar plates or in liquid cultures, may not accurately represent the microenvironment of cells in their natural state. Environmental stresses, due to the lack of proper humidity controls contributing to evaporation and the accumulation of waste by-products in batch cultures, can both increase cell death. The CellASIC™ ONIX Microfluidic Platform overcomes these limitations by enhancing the control of temperature and gas conditions, allowing for flexibility in the addition or removal of different stimuli, and providing a closer mimic of *in vivo* conditions with a perfusion barrier for the diffusion of nutrients, waste and drugs both to and from the cell microenvironment.

In this study, the CellASIC™ ONIX Microfluidic Platform was employed to understand the killing kinetics and mechanisms of action of the synthetic antimicrobial peptides against mycobacteria, and Gram-positive and Gram-negative bacteria. The membrane-impermeable dye, propidium iodide, served as an indicator of bacterial membrane-permeabilization.

A concentration-dependent increase in fluorescently stained bacteria was observed following peptide treatment. At all concentrations tested, the Gram-positive *S. aureus* underwent more rapid membrane-permeabilization as compared to the Gram-negative rod *E. coli*. At 8x MIC, the growth of *S. aureus* and *E. coli* was arrested within 5 min and 25 min respectively. It is likely that this enhanced antimicrobial susceptibility is due to the absence of an outer membrane in Gram-positive bacteria. The rate of membrane-permeabilization for *M. bovis* BCG was far slower, with approximately 50% of bacteria being fluorescently stained after 210 min at 8x MIC. The mycobacterial cell wall consists of an outer membrane with mycolic acids covalently linked to the peptidoglycan-arabinogalactan polymer, and this lipid-rich cell envelope forms a formidable barrier to prevent compounds from readily passing through.

These results highlight the possible membrane-targeted mode of action of the synthetic peptides studied and the feasibility of using microfluidic live-cell imaging to elucidate the antimicrobial mechanisms of AMPs

STUDY OF HEALTHY CARRIERS OF ESBL-PRODUCING ENTEROBACTERIACEAE: ALARMING RATES OF RESISTANCE IN ECUADOR

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Introduction: Animals and humans with and without chronic conditions in the community have been described with ESBL-producing Enterobacteriaceae, furthermore farm animals and pets are increasingly recognized as reservoirs for ESBL-producing strains. Carriage of oxyiminocephalosporin-resistant Enterobacteriaceae in faecal matter among healthy populations has been widely reported. A study published in 2013, identified travel to developing countries as a major risk factor of colonization by multi drug resistant strains (MDR), the authors found after studying faeces of returned travellers, an increased colonization rate of 2.4% before travel to 30% after the travellers return. The study also found that 66% of those colonized on their return had ESBL producing Enterobacteriaceae. The percentage of healthy carriers varies significantly between regions, from <5% faecal carriers in Europe to 69.3% in Asia. The data available from Latin America is exceedingly limited being from only a few countries using small sample sizes, leaving the scientific community blind to the increase of antibiotic resistance in the region.

Aim: The objective of this work is to calculate the rate of healthy carriers in Ecuador, where antibiotic stewardship is non-existent, the public have unrestricted access to antibiotics, and high spectrum antibiotics are regularly used for initial treatment.

Material and Methods: Faecal samples were inoculated in enrichment media with cefotaxime and meropenem (as recommended in the CDC website). After 24 hours, a loop of the liquid media was inoculated in CHROMagar Chromogenic Media with cefotaxime or meropenem. When colonies were isolated, Identification and susceptibility profiles were performed using VITEK®2 compact (bioMérieux, USA) following the manufacturer instructions. Antibiotic susceptibilities were determined by micro-dilution using the GN (21 341), VITEK®2 compact. The criteria proposed by the CLSI 2015. PCR was performed as described in Molecular cloning (M.R. Green and J. Sambrook, Cold Spring Harbor Laboratory Press, 2014) for the genes GES, TEM, SHV, CTX-M-Group 1, CTX-M-Group 2, CTX-M-Group 9. The PCR product was visualized in an agarose gel 0.8%. Purification of the

PCR amplification from the agarose gel, was performed following the manufacturer instructions (Wizard® SV Gel and PCR Clean-Up System, Promega) and was sent to sequence to Macrogen, Korea. Results: From a total of 124 patients, 81 presented at least one ESBL (65.32%), CTX-M-Group 1 the most prevalent (57.03%) follow by TEM (18.52%), CTX-M-Group 9 (11.85%), SHV (8.15%), GES (3.7%) and finally CTX-M-Group 2 (0.74%). Preliminary results have shown the presence of ESBL enzymes in untreated river water and fruit and vegetables from the markets in Quito.

Discussion: The high level of resistance in healthy carriers highlights the necessity to implement an antibiotic stewardship policy at a National level. Medical doctors as well as pharmacists, regularly prescribe antibiotics such as SXT for viral infections (like rotaviruses or enteroviruses) instead of conducting microbiological tests. Within the general population it is common for friends and family to recommend antibiotics for an array of ailments, emphasizing a prevalent lack of comprehension as to the use of antibiotics. It is important to note that not just the abuse of antibiotics but also the use of traditional medicines with antimicrobial activity further exacerbates the proliferation of resistance in the community. The diverse compounding factors have created a selective pressure environment resulting in high antibiotic resistance in the community. The preliminary data implies that the situation is such that in the not too distant future, it is feasible that resistance will be at a level at which antibiotic therapy will no longer be effective. The Ecuadorian government independently certifies drugs allowed in the country; 21 antibiotic therapeutic options have been approved as safe for use in the country and resistance to all of them has been described. In 2014, in a first-level hospital, 55% of *Klebsiella pneumoniae* isolates reported had reduced susceptibility to carbapenems. It is broadly accepted that underreporting is widespread throughout Ecuador and Latin America; the results obtained in this study suggest that antibiotic resistance in Ecuador is at a dangerous level.

Conclusion: The need for antibiotic stewardship in Ecuador is high, as healthy carrier rates of ESBLs is 65.32%. To stem the evolution and hopefully reverse resistance rates in Ecuador, will require a multi-focus approach. Education at all levels is a key starting point, to inform the population of the dangers and futility of antibiotic abuse. Rigorous restrictions of access and usage are crucial, for professionals and public alike, to cease frivolous consumption. Mandatory reporting of all isolated and their susceptibility profiles to a central reference point, where data is analysed and disseminated to the scientific and health professional communities, will help develop strategies in the fight to oppose antibiotic resistance. Finally, it is important to recognize that we live in a global society, where transmission is facilitated by dense populations interconnected by increasingly rapid paths that traverse political borders. It is essential that all participate nationally and internationally in ensuring that a post-antibiotic era is averted.

Poster Presentation Abstracts

Poster abstracts will be finalised weeks before the event

SYNTHESIS OF NEW BIOACTIVE SULPHUR DONOR 4-PROPYL-3-METHYL-1-PHENYL-2-PYRAZOLIN-5-ONE THIOSEMICARBAZONES AND THEIR COPPER COMPLEXES WITH THERAPEUTIC IMPORTANCE

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The emergence of therapeutic drugs limitations such as disease resistance to them and their toxicity effects among others, have continuously interest synthetic, medicinal, inorganic/coordination chemists to develop/design new chemical molecules with novel properties, having broad spectrum therapeutic activities that will be alternative antibiotics. Compounds containing metal centers (metal based therapeutics) have been employed for many years as therapeutic agents because of their biological activities, as such these new organic/inorganic compounds are coordinated with metal ions in an effort to increase their potency. Acylpyrazolones have been employed as pharmaceuticals as well as analytical reagent and their application as coordination complexes with transition metals ion have attracted a lot of research attention. Via a condensation reaction with amines they form a more chelating and superior group of compounds known as Schiff bases. Presented herein is the synthesis of 4-propyl-3-methyl-1-phenyl-2-pyrazolin-5-one thiosemicarbazone and 4-propyl-3-dimethyl-1-phenyl-2-pyrazolin-5-one methylthiosemicarbazone as well as their copper complexes, in continuation of our studies on new bioactive agents that will become potential therapeutics, their effect on coordination with metal ions and a look at drugs combination therapy approach. These synthesized materials have been structurally characterized by means of analytical, spectroscopic, thermogravimetric analysis, as well as x-ray crystallography to establish their identity. Both ligands act as a tridentate ONS molecule, forming a tetrahedral geometry, on coordinating with copper ion. Using the disc diffusion technique, to screen the synthesized compounds at 20 mg/ml against selected bacterial isolates in triplicates, potential bactericidals have been identified at moderate to low activity in relation with standard drug, chloramphenicol. Ligands and

complexes also showed similar antioxidant scavenging properties against 2,2-diphenyl-1-picrylhydrazyl DPPH radical at 0.5mg/ml and 0.25 mg/ml relative to standard Ascorbic acid. With the serial dilution methods, the concentration dependent MIC AND MBC values at different concentrations showed that the metal complexes are more potent with a low MIC AND MBC value of 0.63 mg/ml. As possible antitumour candidates with regards to antioxidant results, anticancer studies of synthesized compounds are ongoing.

STRUCTURAL BASIS OF CARBAPENEMASE ACTIVITY IN THE OXA FAMILY OF CLASS D β -LACTAMASES

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The last decade has seen the evolution of *Acinetobacter baumannii* from a microorganism of low clinical importance into a major nosocomial pathogen. Carbapenem antibiotics have long been drugs of choice for the treatment of *A. baumannii* infections, however the emergence and spread of carbapenem-resistant isolates has greatly reduced their efficacy. The major mechanism of resistance to carbapenems in *A. baumannii* is the production of β -lactamases, and the class D OXA-type carbapenemases of *A. baumannii*, which comprise six families of enzymes (OXA-23, OXA-40, OXA-51, OXA-58, OXA-143 and OXA-235), total over 200 individual enzymes. The ability of OXA enzymes to evolve into a robust carbapenemases as the result of a small number of amino acid substitutions may, in the near future, elevate the ubiquitous enzymes of the OXA carbapenemase families to the status of the most deleterious *A. baumannii* carbapenemases, with dire clinical consequences. We have undertaken the detailed microbiological, kinetic and structural characterization of the plasmid-encoded enzymes OXA-23, OXA-58 and OXA-143, and the chromosomally-encoded *A. baumannii* OXA-51. The three-dimensional structures of these enzymes have been determined to high resolution using X-ray diffraction methods. These results will be presented here.

BACTERIOPHAGE-ANTIBIOTIC SYNERGISM TO CONTROL PLANKTONIC AND BIOFILM PRODUCING CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA

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Pseudomonas aeruginosa (*P. aeruginosa*) is a highly resistant opportunistic pathogen and is capable of forming biofilms on medical devices. Bacterial biofilms, which are micro-colonies encased in extracellular polysaccharide material are so difficult to treat by conventional Antibiotics. During the last decade, *P. aeruginosa* phages have been extensively examined as alternative antimicrobial agents. The aim of the study was to examine the efficacy of phage-antibiotic combination on planktonic and biofilm states of *P. aeruginosa* isolates. In this study, we isolated 6 lytic phages, from hospital effluents, they were tested against 50 *P. aeruginosa* strains, isolated from different clinical specimens delivered to the Diagnostic Microbiology Laboratories, Faculty of Medicine, Alexandria University. Out of the 50 isolates, 15 were susceptible to these phages. So we investigated the biofilm forming capacity of these 15 isolates. The results showed that 14 isolates (93.33%) produced detectable biofilm. The minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC) assays were used to evaluate the antibiotic sensitivity patterns of these *P. aeruginosa* isolates in their planktonic and biofilm phases to Amikacin and Meropenem. Also, the effect of phage on the planktonic and biofilm states of isolates at different multiplicities of infections (MOI) was tested. On the planktonic state, Amikacin-phage combination showed synergistic effect ($P=0.001$), and Meropenem-phage combination showed synergistic effect ($P=0.003$). On the biofilm state, Amikacin-phage combination showed biofilm eradication in 50% of the isolates ($P=0.003$). On the other hand, Meropenem-phage combination showed biofilm eradication in only 14.3% of the strains.

PEPTIDE PGLA REDUCES MULTI-DRUG RESISTANCE IN MDR E. COLI STRAINS

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Antimicrobial peptides (AMP) are small, natural molecules occurring in a wide range of organisms, and play an important role in the innate immune system of vertebrates. AMP can also be found in plants and fungi where they act as a first line defence against bacterial pathogens. AMPs represent a high structural diversity and are considered as promising antibiotic (AB) leads. In this work, we explored the capacity of AMP-AB combinations to restore susceptibility of multi-drug resistant (MDR) *E. coli* strains (i.e. strains that show reduced susceptibility to other antibiotics, after adaptation to increasing concentrations of one single antibiotic) to clinically relevant antibiotics. We evaluated the interaction between AMPs and ABs in both MDR and wild type strains. It was

observed that in 52.23% cases the AMP-AB combination was more effective on the resistant strain than on the wild-type strain. Combinations of PGLA with tetracycline or doxycycline showed strong synergism on the antibiotic resistant strains and this tendency was also observed on an engineered strain carrying a specific multidrug resistance mutation in the *marR* gene. We also observed that sub-inhibitory concentrations of PGLA are able to drastically reduce the MIC of the antibiotic resistant strains, in some cases completely reverting the antibiotic MIC levels to that of the wild type strain. Our experiments suggest that PGLA is a good candidate for further drug development for use in combination with a wide variety of clinical relevant antibiotics, often ineffective due to the emergence of multi-drug resistant pathogens.

DETECTION OF QAC RESISTANCE GENES IN AVIAN PATHOGENIC ESCHERICHIA COLI (APEC)

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Escherichia coli is one of the most important pathogens in the poultry industry. This bacterium is mainly controlled through the use of antibiotics; however, since the restrictions placed on the use of antibiotics in animals, alternatives are needed for the control of bacterial infections. Disinfection can help to control the spread of bacteria. It has been shown that a good disinfection programme for the use in the poultry industry can significantly reduce the clinical signs of bacterial disease. Quaternary ammonium compound (QAC)-based disinfections are used in the poultry industry to control the spread of bacteria. However, some *qac* resistance genes have been isolated and identified, especially in *Staphylococcus aureus*. Some *qac* resistance genes have also been identified in Gram-negative bacteria which include *sugE(c)*, *emrE*, *ydgE/ydgF* and *mdfA*, all of which are chromosome-encoded genes. Other *qac* resistance genes have been identified on mobile elements includes *qacE*, *qacEΔ1*, *qacF*, *qacG* and *sugE(p)*. These genes confer efflux-mediated resistance against QACs. In this study, APECs were screened using PCR for four *qac* resistance genes: *smr*, *qacG*, *qacH* and *qacJ*, which forms part of the Small Multidrug Resistance family.

IN VITRO AUGMENTED PHOTODYNAMIC BACTERICIDAL ACTIVITY OF TETRACYCLINE AND CHITOSAN AGAINST CLOSTRIDIUM DIFFICILE KCTC5009 IN THE PLANKTONIC CULTURES

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Infection with *Clostridium difficile* (*C. difficile*) causes a severe colitis with high recurrence. Treatment of *C. difficile* infection (CDI) is based on antibiotics in spite of the increase of resistance. To interrupt the vicious cycles such as new antibiotics treatment and appearance of resistance strains, photodynamic therapy (PDT) might be a possible alternative therapy for CDI. Tetracycline (TC) has been used as a broad spectrum antibiotic with low risk of CDI and a photosensitizer (PS) in PDT. In vitro PDT against *C. difficile* was conducted using UVA and TC as a PS before in vivo study. To enhance the photodynamic antibacterial activity of TC, we applied chitosan as a boosting agent. Bactericidal effects after PDT, were measured by counting viable cells, DNA damage and membrane integrity. At 1 mg/mL of TC, chitosan treatment combined with PDT, increased the bactericidal effect by >10,000-fold of the effect of PDT alone. Membrane damage and cellular DNA damage demonstrated by EMA-qPCR were also greater in the group treated with PDT + chitosan than in that treated PDT alone. The present study showed that PDT using a combination of TC and chitosan is an effective method for killing *C. difficile*.

THE CRYSTAL STRUCTURE OF THE NISIN RESISTANCE PROTEIN REVEALS THE UNDERLYING MECHANISM OF LANTIBIOTIC RESISTANCE

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Lantibiotics are potent antimicrobial peptides and often considered as next generation antibiotics. Some pathogenic bacteria, however, express membrane-associated resistance proteins, which proteolytically inactivate lantibiotics. The most prominent member of lantibiotics is nisin, which contains five specific and crucial lanthionine rings that are the hallmark for its activity as well as recognition. We report the first three-dimensional structure of the lantibiotic resistance protein superfamily, namely the nisin resistance protein from *Streptococcus agalactiae* (SaNsr) at 2.2Å, which is active against nisin. It contains three domains, a N-terminal helical bundle, a protease cap domain and a protease core domain, which also harbors the highly conserved TASSAEM region. The active center and the binding site of nisin within SaNsr have been characterized via site-directed mutagenesis and molecular modeling, respectively. Due to the presence of the lanthionine rings, nisin itself is highly unsusceptible to proteolytic degradation. However, SaNsr takes advantage of the methyl-lanthionine rings present in nisin for substrate recognition and specificity. This structural information would

pave way for designing small molecular compounds inhibiting antibiotic resistance proteins by which the potency of these fascinating small peptides can be fully explored.

GLOBAL TRANSCRIPTIONAL RESPONSE TO CEFOTAXIME TREATMENT IN CTX-M-1-PRODUCING ESCHERICHIA COLI AND IDENTIFICATION OF VULNERABILITIES IN THE BACTERIUM CAUSED BY CEFOTAXIME TREATMENT

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The worldwide use and misuse of antibiotics have dramatically increased the frequency of resistance among pathogens and has now made antibiotic resistance a demanding and persisting health issue. While much attention has been paid to stress responses in antibiotic sensitive bacteria in the presence of sub-therapeutic concentration of antibiotics, we lack knowledge about adaptive responses in antibiotic resistant bacteria when treated with antibiotics. In this study we look for vulnerabilities that arise in the adaptive pathways in extended spectrum β -lactamase (ESBL)-producing *Escherichia coli*, when they are treated with cefotaxime (CTX). The transcriptomes of two isogenic *E. coli* strains, MG1655/CTX-M-1 carrying blaCTX-M-1 on the chromosome and MG1655/Inc11/CTX-M-1 carrying blaCTX-M-1 on a native Inc11 plasmid, were compared between bacteria grown with and without therapeutically relevant concentrations of CTX. A total of 206 and 804 genes were found to be significantly regulated between the two concentrations in the two strains. KEGG clustering identified 16 functional classes among the significantly regulated genes. In general many genes within translation/ribosomal structure, nucleotide metabolism, carbohydrate transport/metabolism and amino acid transport/metabolism categories were up-regulated. The majority of down-regulated genes were observed in the protein transport, carbohydrate transport/metabolism and amino acid transport/metabolism categories. Protein and peptidoglycan synthesis were upregulated in bacteria treated with CTX, and it was demonstrated that low concentrations of chloramphenicol or D-cycloserine, targeting these systems, strongly reduced MIC of CTX (>32 fold) due to synergy between the drugs. This suggests that when CTX-M-1-producing *E. coli* has an impaired protein or peptidoglycan synthesis it becomes more susceptible to cefotaxime. Inhibition and/or mutations in genes that are central in energy synthesis, purine synthesis, proline uptake or potassium uptake also rendered the resistant bacteria more susceptible to CTX. This knowledge can be used to develop new treatment strategies for cephalosporin resistant *E. coli* by combining the treatment with low concentrations of safe helper-drugs that targets these drug-induced phenotypic adaptations.

DETECTION OF QAC RESISTANCE GENES IN STAPHYLOCOCCUS AUREUS IN THE PRESENCE OF QUATERNARY AMMONIUM COMPOUNDS

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Since restrictions have been placed on the use of antibiotics in animals, alternatives are needed for the control of bacterial infections. Disinfection can help in the fight against bacterial infections, however, if they are not used properly the consequences could be similar to those seen with antibiotic resistance. Disinfectants are being incorporated more in the agricultural industry, especially quaternary ammonium compound (QAC)-based disinfectants, to control bacterial growth in the environment. However, bacteria are capable of acquiring resistance against antimicrobials, including QACs, and can therefore tolerate these harsh conditions. Some low-level resistance has already been observed against QACs, especially in *Staphylococcus aureus*, and some of the genes associated with the resistance have been identified. The level of resistance is still lower than the concentration of QACs used in industry, however exposing microbial communities to sub-inhibitory levels of QACs, could lead to the development of more resistant bacteria. In this study, *Staphylococcus aureus* isolates known to contain individual qac resistance genes were used to screen for the presence of four of the qac resistance genes: smr, qacJ, qacH, and qacG. The *Staphylococcus aureus* isolates were cultivated in the absence and presence of Didecyldimethylammonium chloride (DDAC), which is a fourth generation QAC, after which DNA was extracted and screened for the four qac resistance genes. In the absence of DDAC, only one gene was detected. However, in the presence of DDAC, more than one gene was detected in a particular isolate.

SYNTHESIS AND CHARACTERIZATION OF MOLECULAR ASSEMBLIES OF Ru(II)-Mn(III) AND THEIR ANTI-INFLAMMATORY AND ANTI-COAGULANT EFFECTS TOWARDS PLATELET ACTIVATING FACTOR

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The construction of molecular assemblies containing luminescent metallic complexes which can proceed energy transfer, electron transfer and photocatalysis or photodynamic therapies, with a view to mimicking, at the molecular level, functions performed by natural systems is a topic of growing interest [1]. Among these systems, those based on ruthenium–manganese have attracted a special interest over the last few years [2,3], with the aim to model the function of the donor-side of photosystem II (PSII)[4].

In order to create such polymers, we have performed reactions between the new hexanuclear Mn(III) cage [Mn₆O₂(naph-sao)₆(SCN)₂(EtOH)₆] and the metal-ligand cis-[Ru(dcbpy)₂Cl₂] under different reaction conditions. In the first procedure we study the reaction under irradiation and in the dark in degassed and non-degassed conditions and two polymer complexes in powder form have been isolated. The products of the reactions are characterized by spectroscopy FT-IR & UV-Vis, X-ray power diffraction and cyclic voltammetry.

Moreover, the biological activities of the aforementioned assemblies are tested towards platelet activating factor (PAF)-aggregation under illumination. They are also tested for their ability to modulate PAF-basic metabolic enzyme activities in preparations of rabbit leukocytes. For the first time it is indicated that the synthesized molecular devices of Ru(II)-Mn(III)], with a IC₅₀ of 15 nM is comparable to the widely used PAF receptor antagonists, BN52021 and WEB2170 with IC₅₀ of 30 and 20 nM, respectively, whereas they also affect PAF-catabolism.

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References

- [1] a) L. de Cola and P. Belser, *Coord. Chem. Rev.*, 1998, 177, 301. b) M. Kaplanis, G. Stamatakis, V. D. Papakonstantinou, M. Paravatou-Petsotas, C. A. Demopoulos, C. A. Mitsopoulou, *J. Inorg. Biochem.*, 2014, 135, 1
- [2] R. Lomoth, A. Magnusson, M. S. Odin, P. Huang, S. Styring and L. Hammarstroem, *Photosynth. Res.*, 2006, 87, 25.
- [3] D. Burdinski, E. Bothe and K. Wieghardt, *Inorg. Chem.*, 2000, 39, 105.
- [4] (a) B. Loll, J. Kern, W. Saenger, A. Zouni and J. Biesiadka, *Nature*, 2005, 438, 1040; (b) M. Haumann, P. Liebisch, C. Müller, M. Barra, M. Grabolle and H. Hau, *Science*, 2005, 310, 1019.

EFFECT OF RHAMNOLIPIDS ON TRICHOSPORON BIOFILM IN FLOW-CELLS

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Biofilm are clusters of single or mixed cell population which is often found attached to abiotic or biotic surfaces in the environment. Cells in the biofilm are protected by self-produced extracellular matrix which provides, besides other, protection against external effects. Biofilm formation, in the case of pathogenic microorganisms, is undesirable state because of increasing resistance to antibiotics and other antimicrobial agents. Therefore, the current research is focused on the development of new anti-biofilm strategies. One possible way how to disrupt and prevent the biofilm formation can be application of biosurfactants as surface active agents. In addition many biosurfactants have antimicrobial activities. Moreover, unlike synthetic surfactants, these compounds are biodegradable and less toxic which is considerable advantage. Here we present eradication of *Trichosporon cutaneum* biofilm by application of four rhamnolipid mixtures of various compositions and properties. Screening tests were carried out in static condition with biofilm formed on glass surface. In these conditions were removed over 50% of biofilm (24 h growth) by all tested rhamnolipids in concentration 100 mg/l during 16 hours action. In addition, the use of rhamnolipids in concentration 500 mg/l led to removal of more than 80% of biofilm in the same time frame. In dynamic conditions carried out in flow chamber three rhamnolipid mixtures were capable disrupt over 70% of biofilm by 2 hours action and more than 85% by 16 hours. From our results, rhamnolipids seem to be a promise tool for the effective disruption of mature biofilm.

CORRELATION BETWEEN GENOTYPIC AND PHENOTYPIC TEST IN MYCOBACTERIUM TUBERCULOSIS FROM ECUADOR

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Introduction: The spread of drug-resistant Mycobacterium tuberculosis has become a public health concern. Early detection of resistance is necessary to avoid failed treatments. The sequencing of genes related to resistance is an alternative to achieve rapid resistance detection. The aims of this study were to establish the relationships between the phenotypic resistance profile and the related gene mutations; and to identify the most common mutation associated with drug resistance in M. tuberculosis isolated in Quito-Ecuador.

Methods: 67 M. tuberculosis isolates proceeding from the bacteria collection of the Microbiology and Tuberculosis department of Vozandes-Quito Hospital, were analyzed: including forty eight (71.64%) MDR isolates, fourteen (20.89%) INH-mono-resistant isolates and five (7.46%) RIF-mono-resistant isolates. To determine mutations related to RIF rpoB gene were sequenced for INH resistance, the katG gene and inhA promoter region were sequenced.

Results: Fifty-three RIF-resistant isolates were analyzed, of which 90.6% (48/53) had at least one mutation in rpoB, whereas five (9.4%) resistant isolates lacked these mutations. Sixty-two INH-resistant isolates were analyzed, of which forty-seven (75.8%) had a mutation in the katG and/or inhA promoter. Of the forty-seven isolates, two (3.3%) had a mutation in both loci; while thirty-four (54.8%) presented mutations only in katG, and eleven (17.7%) showed mutations only in inhA promoter. Fifteen (24.2%) of the sixty-two INH-resistant isolates lacked the mutation in both loci. Eight profiles of mutation were recorded for rpoB gene, where S531L (58.5%) was the most frequent. For the katG gene were recorded five mutation types, S315T (50%) was mostly registered. Only the -15 mutation in inhA promoter were found.

Conclusion: This study shows that mutations in rpoB are highly associated with RIF resistance, so that the sequencing of this gene allow us early detection of RIF-resistant isolated, as reported in other studies. katG and inhA promoter mutation are useful as predictive markers of isoniazid resistance. However, is necessary to have the phenotypic resistance profile because several studies propose that up to 25% of the INH-resistant isolates did not have mutations in either katG and inhA promoter.

IDENTIFICATION OF PLASMID-ENCODED SMALL RNA IN A MULTIDRUG-RESISTANT PLASMID pNDM-HK CARRYING blaNDM-1

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Small RNAs (sRNAs) regulated gene expression is one of the emerging post-transcriptional regulatory mechanisms in bacteria and it plays a key role in response to physiological stimulation in bacteria. The first plasmid-encoded sRNA was identified from *Escherichia coli* plasmid and found to modulate plasmid replication and segregational stability.

In this project, we set out to identify novel sRNA in an emerging multidrug resistance IncL/M plasmid pNDM-HK through high throughput sequencing. Numerous antibiotic resistance genes are found in pNDM-HK, including New Delhi metallo- β -lactamase 1 (NDM-1), a carbapenemase that causes worldwide health threat in recent years. Intriguingly, six novel sRNAs located at different regions of plasmid such as replication, stability and variable regions, were identified from pNDM-HK. The sRNA-phylogenetic tree that was built based on these novel sRNAs provided unexpected but significant information about the evolutionary pathway of pNDM-HK plasmid including the possible genes acquisition and insertion from the relevant plasmids. In addition, the sRNA-phylogenetic tree can specifically cluster the IncM2 type and distinguish from other IncL/M plasmids. Moreover, we also characterized one of the plasmid-encoded sRNAs, pNDM-sR3 down-regulate genes for DNA replication in an Hfq-dependent manner, facilitating the fitness and circulation of drug resistance plasmid in bacteria. In conclusion, this is the first study to systematically identify and characterize sRNAs in the clinically-isolated multidrug resistance plasmid, pNDM-HK. We believe these novel sRNAs could provide further information on dissemination and maintenance of the multidrug resistance plasmid, which can be adopted as therapeutic targets in the future.

CHARACTERISATION OF A CLONE OF SALMONELLA ENTERICA SEROTYPE ENTERICA SEROVAR INFANTIS THAT HARBOUR CTX-M-65 IN ECUADOR

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Introduction: In developing countries, food hygiene regulations are difficult to enforce, as a consequence food transmitted diseases present a big problem, exacerbated due to the lack of reporting. This study reports a circulating clone, the first Salmonella spp resistant to cephalosporins described in Ecuador. The clone has been circulating since January 2014 yet has only been reported 5 times.

Methodology: We performed PCRs to identify the mechanisms of resistance; ERIC-PCR was used to identify clonality. Biofilm and transformation assays were conducted to determine survivability and resistance gene location respectively.

Results: PCR results confirmed that all 5 isolates collected over the study period were the same clone of Salmonella Infantis. The clone was found to harbour CTX-M-65, the gene responsible for resistance to cephalosporins and results obtained suggest that the enzyme is chromosomal. Biofilm assays revealed that the clone strain's ability, in environmental conditions, to produce biofilm is double of that found in the sensitive control strain.

Conclusion: The clone strain of Salmonella infantis that harbours CTX-M-65 was the first isolated in Ecuador and has been present in Quito for a minimum of 1.5 years. The clones ability to out produce sensitive strains biofilm production in environmental condition may be the reason for the clones longevity, but the low number of isolates suggests that under-reporting could hide indicators of an on-going outbreak. The lack of implementation of Food hygiene is increasing the number of Food transmitted diseases every year. A good reporting system is needed as most of the laboratories do not study microorganisms like Campylobacter or Salmonella due to the lack of resources and as a consequence they are not reported leaving blind the scientific community.

ANTIMICROBIAL EFFECT OF METAL NANOPARTICLES

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It is well known that microorganisms in the biofilm are more resistant to antibiotics and other environmental stresses than their planktonic counterparts. Therefore, they are one of the main causes of development of chronic infections. Their ability to resist antibiotics is influenced by number of factors, including the poor penetration of the antibiotics into the biofilm matrix, the particular microenvironment and/or the adaptive mechanisms of the microorganisms themselves. Due to increased resistance to antibiotics and the current lack of effective antibiotics, new options are needed to control biofilm related infections. One promising approach involves use of metal nanoparticles (NPs) which show antimicrobial and antibiofilm activities.

The aim of this study was to compare use of the metal NPs (gold, silver; both with diameter 20 nm) against planktonic cells and for biofilm treatment formed by *Pseudomonas aeruginosa*, *Candida krusei* and *Candida parapsilosis*. Potential antimicrobial/antibiofilm activity was tested by adding the metal NPs for 24 hours. The effect of NPs against planktonic cells was determined using microcultivation device Bioscreen C and against biofilm using crystal violet staining and an automated cell imaging system CellaVista.

NANOPARTICLES AS A NEW TOOL OF ANTIMICROBIALS

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Because of serious problems associated with the growing number of antimicrobial-resistant pathogens, the emphasis is placed on the research and development of new effective drugs. Microbial resistance can be described as the process of evolution in a response to selective pressure by antimicrobials. Therefore, there are arising concerns about the application of new antimicrobials which can solve contemporary problems, but also can lead to the development of new resistant strains. Consequently, entirely new approaches to antimicrobials development are necessary.

Use of nanotechnology in immunization, design and delivery of antimicrobial drugs has been explored as a promising alternative to traditional care. Antimicrobial activity has been described for variety of nanoparticles, e.g. nano-Ag, nano-ZnO, nano TiO₂. The dimension of nanoparticles, ranging between 1 and 100 nm, determines their unique chemical and physical properties, which are different from that of their bulk counterparts, and also influence their biological activity. The mechanism of nanoparticles action is very complex and has not been clarified in all details. One of the main manifestations of nanoparticles action seems to be an ability to induce oxidative stress in treated cells. Moreover, because of their nano-size, nanoparticles readily penetrate into cell cytosol, where they can react with cell components and thus negatively influence cell processes. Given that the nanoparticles can affect cell viability at many sites simultaneously, the development of nanoparticle-resistant microorganisms is much less probable.

The extent of nanoparticles impact on cell viability depends on many factors, such as type and size of nanoparticles, surface modifications, but also type of microorganism and its physiological state. Therefore, for the potential application of nanoparticles as antimicrobials, the investigation of interactions between the nanoparticles and the microorganism is crucial. In the present study, we examined antimicrobial capacity of different nanoparticles against model prokaryotic and eukaryotic microorganisms. We focused not only on cell viability, but also on some aspects of the role of oxidative stress in nanoparticles toxicity.

IN VITRO TOXICITY ASSESSMENT OF ANTHRAX TOXINS FOR INTRACELLULAR DRUG DELIVERY

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Many pharmaceutical agents need to be delivered intracellularly to exert their therapeutic action. Due to biological membranes preventing large hydrophilic molecules, such as siRNA agents or antisense oligonucleotides from spontaneously entering cells, new mechanisms to carry proteins across membranes into the cytosol of mammalian cells have been developed to overcome the pharmacokinetic and toxicity problems.

One such drug delivery technology is based upon the anthrax toxins ability to delivery macromolecules to the cytosol. A detoxified version of anthrax toxin includes the proteins protective antigen (PA) and the N-terminal lethal factor (LFn) which has the potential to deliver a variety of pharmaceutical forms. In this study, the toxicity of PA and LFn fused in frame to markers such as green fluorescence protein (GFP) or the DNA binding protein GAL4 was assessed using the MTT cell viability assay over different concentrations of proteins (from 0.1 to 200 (µg/mL)) in HeLa cells. The PA produced a marked effect over higher concentrations. LFn-GFP lacks any toxic effect when tested over three days and provides opportunity to use it as a protein carrier without any limitation in toxicity. This is confirmed by testing PA and LFn- GFP together and it shows results close to those of PA individually. Other suggested combination of LFn and GAL4 (LFn-gal4), that may applicable in gene delivery techniques, obtain an occurrence of developmental abnormalities to cells but still represent an applicable method as all readings above IC₅₀. The combination of PA, LFn-GAL4 in addition to ASO, which may be a powerful tool in gene therapy applications, has shown a satisfactory out-put of safety with minimal toxicity effect as the IC₅₀ was above the maximum concentration tested 200 (µg/ml). Further investigation in vivo trials is warranted into the safety and efficiency of this novo intracellular delivery technology.

ASSESSING THE PREVALENCE OF TETA IN A COMMERCIAL PIG FARM

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Most studies on the distribution of resistance genes within a farm and the transmission to the environment rely on traditional (culture-based) microbiological methods. In this study, molecular biological methods were used to determine the prevalence of antibiotic resistance genes at a farm in preparation of a feeding trial. Various samples were taken and analyzed for bacterial load and tetracycline resistance. Samples were taken in a farrowing pen in a commercial farm close to Nitra, Slovakia. Swap samples were taken from piglet and sow feces, water and feeding troughs, the walls and from dust and spider webs. Two types of swabs were used: dry swabs and swabs with Amies medium. To develop suitable DNA-extraction methods, procedures already described in literature were employed. Swab samples were extracted with the Sodium Hydroxide HotShot method (Truett et al., 2000). Additionally, liquid samples of sow colostrum were taken and extracted with either the Chelex100 method (Sweet et al., 1996) or the Fast DNA Spin Kit (MP Biomedicals). DNA was amplified by the KAPA SYBR Green assay using primers for either 16S-rDNA (Clifford et al., 2010) or tetA (Ng et al., 2001). TetA was detected in all tested samples, not only in animal feces but also throughout the farm building. Interestingly, tetA was detectable even in dust and spider web samples. Molecular determination of antibiotic resistance genes is a fast method that allows not only detection but also quantification of resistant bacteria and will therefore help to identify the risk of resistances spreading into the environment.

TWENTY YEARS OF CLINICAL STUDIES ON ANTI-PSEUDOMONAS IgY TO CYSTIC FIBROSIS PATIENTS.

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Objectives: Clinical studies have been running in Sweden for twenty years with CF-patients on Anti-pseudomonas IgY (Anti-PA IgY) to prevent infections with Pseudomonas aeruginosa (PA) in order to find out efficacy and adverse events. The promising results have given Anti-PA IgY an Orphan drug designation and are the background for a Phase III study started in 2010.

Methods: Two studies to register efficiency and adverse events have been running following each other. First phase II study 1995 -2002: Two groups with intermittently PA-infected patients - one treated with Anti-PA IgY, the other group (in Aarhus) without IgY treatment. Microbiologists did not know which group they analyzed. Second prolonged study 2002 until now: Anti-PA IgY achieved certificate to 20 patients in 2002. Followed in the same way as the first study but without control group.

Two CF women continued with Anti-PA IgY during pregnancy. One was pregnant twice. One boy transplanted 12 years ago due to infections with PA and atypical mycobacterium.

Results: First study: Group with Anti-PA IgY: 2.35 positive PA cultures/100 months; Untreated group: 7 positive PA/100 months. The duration from first to second colonization with PA was significantly prolonged for the treated versus the control group (Kaplan-Meier $p=0.015$). The time from first PA infection until chronic infection occurred was prolonged in the Anti-PA IgY treated group. The time until PA was transformed to the severe mucoid form was prolonged. The few infections in treated group minimized the need for antibiotics. Lung function and BMI were well preserved. Prolonged group: similar effects as those in the first study. Totally 33 patients have been included in the two studies. Together they have gargled once daily for more than 250 years (~100.000 doses). There have been no adverse events reported. All pregnancies carried out well and gave birth to three healthy babies. Transplanted pat has hitherto not had any new pseudomonas or atypical mycobacterium infections after transplantation.

Discussion: Anti-PA IgY has shown good results both in efficiency and absence of adverse events. It reduces the use of antibiotics and thus also the risk of resistant bacteria. The gargling is convenient to use. The treatment is cost effective. The cost for Anti-PA IgY is much less than the costs for antibiotics. In addition, the costs for days of illness and for hospitalization will be much lower.

Conclusion: Hopefully the now running double-blind, randomized phase III study will give results as above and Anti-PA IgY might thereafter be registered and physicians will be able to give anti-PA IgY to all eligible patients.

MULTI DRUG RESISTANT ESBL E. COLI FROM CATTLE SLURRY IN A DAIRY UNIT IN NOTTINGHAM

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ABSTRACT

A significant amount of antibiotics is used in treating infections in farm animals, and as prophylactics against infection. Antimicrobial resistance is a crucial problem that is now of great concern in public health, with food and food producing animals as a potential route for spread of these resistances, especially resistance to cephalosporins which is increasing. In this study E. coli isolated from farm cattle slurry was used as an indicator for the persistence of antibiotic resistance genes in the environment. TBX agar, MacConkey Agar with and without Cefotaxime (2mg.L-1) and CHROMagar ESBL medium were used to isolate and enumerate the total coliforms, and E. coli from slurry samples collected from different areas in a Dairy Research Unit. 160 isolates from TBX plates were confirmed as E. coli by phenotyping (indole, oxidase and API20E). ERIC PCR was used to investigate the genotypic diversity of these isolates. Antibiotic sensitivity testing using the disc diffusion method was used to investigate the antibiotic resistance profile of E. coli isolates to a range of antibiotics (17 antibiotics). A confirmation for the ESBL/AmpC multiresistance E.coli was done using total ESBL/AmpC confirmation kit. PCR was also used to detect ESBL and AmpC genes CTX-M, SHV, TEM and CMY. More than 55% of the isolates showed multiple resistances to the tested antibiotics, with isolates resistant to between 2 and 13 antibiotics. The highest percentage of resistance was to Ampicillin and the lowest resistance showed against Imipenem . In respect to Extend Spectrum Cephalosporins, the highest resistance was against Cefotaxime.