MICROBIOLOGY SOCIETY

FOCUSED MEETING
2017

Antimicrobial Resistance and One Health

Poster Abstract Book

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Focused Meeting 2017: Antimicrobial Resistance and One Health

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Poster number: 1

Prevention of antibiotic resistance horizontal gene transfer on touch surfaces

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Abstract

Horizontal gene transfer (HGT) conferring resistance to many classes of antimicrobials has resulted in a worldwide epidemic of nosocomial and community infections caused by multidrug-resistant microorganisms, leading to suggestions of returning to the pre-antibiotic era. Whilst studies have focused on HGT *in vivo*, this work investigates whether the ability of antimicrobial resistant pathogens to persist in the environment, particularly on touch surfaces, may also play an important role. Escherichia coli clone ST131 and Klebsiella pneumoniae harbouring extended-spectrum--lactamase (ESBL) blaCTX-M-15 and metallo--lactamase blaNDM-1, respectively, showed prolonged survival on stainless steel, with approximately 10e4 viable cells remaining from an inoculum of 10e7 CFU per cm2 after 1 month at 21°C. HGT of bla to an antibiotic-sensitive but azide-resistant recipient E. coli strain occurred on stainless steel dry touch surfaces and in suspension but not on dry copper. The conjugation frequency was approximately 10 to 50 times greater and occurred immediately, and resulting transconjugants were more stable with ESBL E. coli as the donor cell than with K. pneumoniae, but blaNDM-1 transfer increased with time. Rapid death, inhibition of respiration, and destruction of genomic and plasmid DNA of both pathogens occurred on copper alloys accompanied by a reduction in bla copy number. Naked E. coli DNA degraded on copper at 21°C and 37°C but slowly at 4°C, suggesting a direct role for the metal. Therefore, copper alloys could be useful in the prevention of infection spread and gene transfer in the healthcare and public transportation environments, particularly where cleaning and disinfection practice is not 24/7.

Poster number: 2

Borrelia bacteriophages for diagnosis and treatment of Lyme Disease and Relapsing Fever

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Abstract

Bacteriophages (phages) are viruses that infect bacteria. They have been investigated for diagnosis and treatment of many types of bacterial infections. A very few studies have been carried out on phages that infect *Borrelia*, a group of Spirochaetal bacteria that are the causative agents of Lyme Disease (LD) and Relapsing Fever (RF). The disease is transmitted to humans through the bite of infected ticks and louses. There are concerns about the rise of antibiotic resistant *Borrelia* strains. Up-to-date, the diagnosis and treatment options for LD and RF are problematic. In this project, we aim to isolate and characterise *Borrelia* phages to obtain a better understanding of their biology and improve both diagnostics and therapeutics.

Data are presented within the following three strands:

1. Phage-based diagnostics for detecting *Borrelia* was developed and validated. The performance of the LD assay was examined against 222 patient samples and showed an overall sensitivity of >90% and a specificity of 100%. This method can distinguish LD and RF and has the potential of identifying active *Borrelia* infection.
2. A national tick collecting network has been established throughout the UK, from which a screening strategy has been developed for detecting lytic *Borrelia* phages. In addition, novel phage particles were observed from Mitomycin C-treated *Borrelia* cultures.
3. We have identified a pair of *Borrelia* phage-encoded enzymes, which were predicted to break down bacterial cell walls. Both enzymes have been manufactured in a yeast protein expression system and are currently under investigation of their *in vitro* 'anti-*Borrelia*' properties.

Poster number: 3

Resistomics of sputum samples from chronic obstructive pulmonary disease

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Abstract

The prevalence of antimicrobial resistance (AMR) genes is a major global threat. While the human gut microbiota has been extensively studied, little is known about the bacterial community in the lower respiratory tract as a reservoir and source of AMR genes. Chronic Obstructive Pulmonary Disease (COPD) is a common respiratory condition associated exacerbations that are usually treated with antibiotics. We studied the prevalence of AMR genes in COPD sputum samples.

Using high-throughput quantitative PCR targeting 296 specific AMRs, we determined the prevalence of AMR genes in DNA extracts from 195 well-characterized sputum samples from patients with COPD (n=165) and induced samples from age matched (45-71) healthy control subjects with known antibiotic histories for the past 12 months (n=30). Culture of the COPD samples revealed very low frequency of phenotypic resistance in respiratory pathogens. Of the 245 AMR genes detected in, antibiotic deactivation and efflux pumps were dominant and the 10 most prevalent were *mefA*, *matA/mel, sulA/flop* and *tetM* (>90%), *cfxA* and *pmrA* (>80%), *tetQ, fabK, ermB* and *IS613*(>60%). The same prevalence was observed in the age matched healthy control samples.

AMR genes are frequently found in airway samples, prevalence did not increase in COPD vs age matched controls nor did it relate to any assessed patient characteristics. Does the human respiratory tract collect AMR genes in an age related fashion?

Poster number: 4

Effect of yeast cell wall mannan-rich fraction on antibiotic resistant Enterobacteria

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Abstract

Antibiotic resistance has the potential to become one of the greatest problems of our generation. Issues related to antimicrobial resistant bacteria in Europe are estimated to cost about €1.5 billion per year, as once treatable diseases are becoming incurable. Producers are under pressure to reduce their antibiotic load; thereby alternative strategies that promote animal health without negatively affecting the world's food supply are required. The focus of this research is to assess the role of mannan rich fraction (MRF) in mitigating antibiotic resistance in drug resistant Enterobacteriaceae. The effect of MRF on *E. coli* and *Salmonella* strains was assessed by monitoring microbial growth in the presence of antibiotics, with or without MRF. Two strains of *E. coli* showed a statistically significant increase in sensitivity to antibiotics in the presence of MRF (0.5% w/v). Growth of extended-spectrum beta-lactamase (ESBL) producing *E. coli* was reduced by 21% ($p \leq 0.05$) in the presence of cefotaxime (4.5 μgmL^{-1}). Growth of ampicillin resistant transformed *E. coli* in the presence of ampicillin (45 μgmL^{-1}) was reduced by 43% ($p \leq 0.05$). MRF was also observed to induce an increase in sensitivity of multi-drug resistant (MDR) *Salmonella enterica* serovar Dublin to ticarcillin and piperacillin (45 μgmL^{-1}), by 19% and 21% respectively. The results show the ability of MRF to revert the resistance of certain Enterobacteriaceae to a more drug sensitive state. These results may be beneficial in the search of alternate strategies to promote animal health without contributing to the growing issue of antimicrobial resistance.

Poster number: 5

Identification of *Staphylococcus aureus* factors that promote adherence to atopic dermatitis skin

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Abstract

Staphylococcus aureus colonises the skin of over 90% of atopic dermatitis (AD) patients, while skin carriage of the organism by healthy people is less common. AD is a common childhood skin disease affecting more than 20% of the population in the USA and Europe. Colonisation of AD skin by *S. aureus* exacerbates disease symptoms. *S. aureus* adheres to dead flattened cells (corneocytes) in the upper layer of the stratum corneum. The structure and protein composition of corneocytes in AD skin is different to corneocytes from healthy individuals. Elucidating the molecular basis of the interaction between *S. aureus* and the skin is critical to understanding pathogenesis and in forming targeted therapies to reduce colonization and infection in AD patients. The objective of this study was to identify bacterial proteins that promote attachment of *S. aureus* to AD corneocytes. We studied primary clinical strains of *S. aureus* and isogenic mutants deficient in specific adhesins. The *S. aureus* cell wall anchored surface proteins, clumping factor B (ClfB) and fibronectin binding proteins A and B (FnBPA, FnBPB) were found to promote attachment of *S. aureus* to purified corneocyte proteins. A ClfB-deficient mutant had reduced ability to adhere to corneocytes taken from AD patients. In summary this work describes adhesive interactions that may facilitate adherence of *S. aureus* to AD skin, and thus provides important new insights into the first step in the establishment of *S. aureus* skin infection in AD patients.

Poster number: 6

The agri-food chain as a reservoir for antimicrobial resistant *Escherichia coli*

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Abstract

Antimicrobial resistance is recognised globally as a major public health concern. There is currently insufficient data in Ireland and elsewhere on the presence of antimicrobial resistant *Enterobacteriaceae* (AMR-E) in the agri-food chain to support an assessment of risk associated with human infection. The aim of this study was to assess chicken caeca for the presence of AMR-E.

Overall, 36 chicken caeca samples were obtained from the Agri-Food and Biosciences Institute, Belfast (12 each from 10, 21 and 34 day old chicks), between March-April 2016. Samples were screened for the presence of; carbapenemase-producing *Enterobacteriaceae* (CPE), extended spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL-PE) and fluoroquinolone resistant *Enterobacteriaceae* (FQR-E). Susceptibility to 14 antimicrobials was examined in accordance with EUCAST criteria. Isolates identified phenotypically as ESBL producers were examined for the presence of $bla_{\text{CTX-M-group 1}}$, $bla_{\text{CTX-M-group 2}}$ and $bla_{\text{CTX-M-group 9}}$ encoding genes by real-time PCR. *E. coli* isolates were classified into phylogenetic groups by multiplex PCR.

All 36 samples were positive for the presence of ESBL-producing *E. coli*, all of which screened positive for $bla_{\text{CTX-M-group 1}}$ and were classified into phylogenetic group F. A total of 23/36 (64%) of the caeca samples contained FQR-E (all *E. coli*). FQR-E isolates were classified into phylogenetic group B1. No CPE were detected.

The similarity of the isolates, particularly the ESBL-producing *E. coli*, suggests dissemination of a common strain of *E. coli* and the possibility of vertical transmission among poultry. These findings highlight the need for increased surveillance of the presence of antimicrobial resistance in the agri-food chain.

Poster number: 7

Indistinguishable NDM-Producing *Escherichia coli* isolated from recreational waters, sewage and a clinical specimen in Ireland

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Abstract

The rapid dissemination of New Delhi metallo-beta-lactamase (NDM)-producing *Enterobacteriaceae* is of major concern worldwide. The potential for recreational water to contribute to the spread of antimicrobial resistance (AMR) has not been well studied. The aim of this study was to examine freshwater inflows into recreational bathing seawaters for carbapenemase producing *Enterobacteriaceae* (CPE).

Thirty litres of freshwater from two streams (A and B) flowing across a bathing beach were collected on seven dates between May-September 2016. Samples were filtered using a large volume filtration system. In September 2016, 250ml samples were collected from three nearby sewage points. Samples were screened for CPE using Brilliance CRE agar. Susceptibility testing was carried out using EUCAST disc diffusion methods and criteria. Carbapenem resistant isolates were examined for CPE encoding genes by real-time PCR. Confirmed CPE were examined by pulsed field gel electrophoresis (PFGE). Clinical CPE isolates from The National Carbapenemase Producing *Enterobacteriaceae* Reference Laboratory Service were examined for relatedness to environmental isolates.

NDM-producing *Escherichia coli* were identified in 2/7 freshwater (stream B) samples, and 3/3 sewage samples. Isolates had identical antibiograms and were indistinguishable from each other and from a clinical NDM-producing *E. coli* by PFGE. NDM-producing *Klebsiella pneumoniae* were identified in 2/3 sewage samples. Isolates appear closely related to each other and to a clinical NDM-producing *Klebsiella pneumoniae* by PFGE.

These findings reveal the potential for human faecal contamination of the aquatic environment to contribute to rapid community dissemination of CPE in addition to other long established risks of discharge of untreated sewage.

Poster number: 8

Tracking plasmid-mediated antibiotic resistance from environmental reservoirs to the food chain

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Abstract

Antibiotic resistance (AR) is currently the greatest threat to animal and human health, as stated by the World Health Organisation. AR bacteria can occur naturally in the environment including in soil, water and organic fertilizers used in agriculture. It is assumed that the AR can pass from these sources to humans via plant-based food. However, there is currently little known on how the resistance is transferred from these environments to commercially grown plants. This study focuses on lettuce cultivation and determining the sources from which the AR is transferred to the plants. Plasmids will be extracted from soil, irrigation water and lettuce samples. Antibiotic profiling and metagenomics will be carried out to determine which plasmids are involved in the transfer of AR at the interfaces. This work will inform on the plasmid-mediated AR present in these environmental reservoirs whilst also highlighting what roles manure and water application play in influencing the type and frequency of transferred AR. This information will play a vital role in the identification of environmental reservoirs of AR and will provide a stepping-stone to mitigate the transfer of AR to humans through the food chain.

Poster number: 9

The nucleotide salvage pathway is intrinsically involved in beta-lactam antibiotic resistance in *Staphylococcus aureus*

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Abstract

The salvage pathway is used to recycle nucleotides and plays an important role in the degradation and synthesis of DNA. By salvaging nucleotides, the cell saves energy on *de novo* synthesis, and on importing external nucleotides. We have identified a nucleoside phosphorylase mutation in the purine salvage pathway that is associated with increased beta-lactam resistance. This enzyme converts intracellular guanine derivatives i.e. deoxyguanosine triphosphate and cyclic guanosine monophosphate, into a common precursor, guanine. Guanine is then re-phosphorylated to guanine monophosphate. We hypothesise that alterations in the intracellular guanine pool may trigger cell-wide effects via the secondary nucleotide messengers ppGpp and cyclic di-adenosine monophosphate (c-di-AMP). These data link the purine salvage pathway and beta-lactam resistance in *S. aureus* and our ongoing experiments are investigating potential interaction between purine metabolism and cell wall biosynthesis pathways, and antibiotic resistance.

Poster number: 10

Selection for clinical resistance at environmentally relevant concentrations

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Abstract

The Minimum Selective Concentration (MSC) is the lowest antibiotic concentration that can select for antimicrobial resistance (AMR). MSC data, particularly in complex communities, is severely lacking. This data is crucial for assessing the ability of antibiotics to select for resistance in the environment, a risk currently unconsidered in standard environmental risk assessment.

In this study, a natural complex community (untreated waste water) was evolved in the presence of cefotaxime, the WHO 'critically important' human medicine, in a daily transfer experiment. Metagenomic analyses of select assay concentrations at the end of the community experiment were performed to assess the level of co-selection for other resistance determinants. Within the beta-lactam class of resistance determinants, the clinically important CTX-M genes were shown to be preferentially enriched over all other beta-lactam resistance genes. Therefore, CTX-M genes were targeted with qPCR to calculate an MSC.

Results show the MSC of cefotaxime was low: 30µg/L. This suggests selection for CTX-M genes may be occurring in some aquatic systems, based on previously measured environmental concentrations. Metagenomic analyses revealed that several AMR gene classes were also enriched by cefotaxime exposure, including aminoglycoside, sulphonamide and trimethoprim resistance genes. The observed preferential selection of CTX-M genes at both low and high concentrations may explain the rapid spread of these genes worldwide, and their presence in the environment.

Together, these findings indicate selection for AMR may be occurring in the environment. Further work should continue to generate MSCs for other antibiotics in complex communities, to aid design of appropriate mitigation strategies.

Poster number: 11

Characterisation of a ciprofloxacin resistance plasmid isolated from the gastrointestinal tract of broiler chickens

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Abstract

Plasmid-mediated antibiotic resistance (AR) is a major animal and human health problem. Many plasmids have the ability to transfer between different bacterial species. This is a threat to human and animal health if a plasmid carrying a resistance gene is transferred to commensal bacteria or clinical pathogens. This study focused on investigating plasmid-mediated antibiotic resistance in the caecal bacteria of broilers, which are raised for meat production.

We identified a multi-drug resistant plasmid from the caecal bacteria of broilers via a direct extraction and plasmid amplification method. The plasmid, which was transformed into *Escherichia coli*, confers high levels of resistance to four different antibiotics: ampicillin, tetracycline, kanamycin and ciprofloxacin. Antibiotic susceptibility testing was performed on the transformant using disk diffusion and agar dilution methods. The resistance to ciprofloxacin is of particular interest, as a disk diffusion assay revealed no zone of inhibition around a 5µg ciprofloxacin disk and the transformant has a minimum inhibitory concentration to ciprofloxacin of 128 mg/L. The clinical breakpoint for ciprofloxacin resistance is 4 mg/L according to the CLSI guidelines. PCR testing will identify the genes responsible for conferring resistance and the conjugative ability of the plasmid will be assessed.

This raises concerns over both disease control in animals and food safety, as there is a possibility of the transfer of resistance to humans through food. The plasmid may also have the ability to transfer to other pathogenic Enterobacteriaceae. Ciprofloxacin is a clinically relevant broad-spectrum antibiotic that is used to treat both gram-positive and gram-negative infections.

Poster number: 12

The antibiotic susceptibility of faecal coliforms isolated from wastewater plant effluent

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Abstract

The discovery of antibiotics has had a significant impact on the control of infections caused by bacteria. However, the development of antibiotic resistance bacteria (ARB) has led to the failure of antibiotic treatment. The objective of this work is to identify the antibiotic resistance prevalence of faecal coliforms isolated from wastewater treatment plant (WWTP) effluent samples.

Samples were collected from two WWTPs (A and B) in 2015 and 2016. The bacterial enumeration and isolation were assessed by membrane filtration method. The minimal inhibitory concentrations to nine antibiotics of isolated bacteria were determined according to the CLSI guidelines.

WWTP A effluent samples contained an average of 379 CFU/mL of faecal coliforms. From WWTP B about 476 CFU/mL of faecal coliforms were enumerated. A set of 498 faecal coliform strains were isolated from effluent samples of both WWTPs. Among all tested antibiotics, β-lactam antibiotic (ampicillin and amoxicillin) resistance prevalence (> 80%) was the highest from both WWTPs, followed by tetracycline, trimethoprim, ciprofloxacin. Colistin resistant bacteria were found at 32.5% on average in WWTP B but not in WWTP A (10%). The other resistance phenotypes were observed at a moderate-low rate. Multidrug resistant bacteria (MDRB) were found at high percentages in all sampling periods (>50%).

ARB isolated from WWTP effluent represent a serious threat for human health as they can serve as a source for maintenance and dissemination of antibiotic resistance genes. Our results will provide important information for future research on the spread of antibiotic resistance from WWTPs to the environment.

Poster number: 13

Cooperation, competition and antibiotic resistance in bacterial colonies

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Abstract

The evolution of antibiotic resistance is one of the major challenges facing society. Much of research into antibiotics focuses on solitary cells, swimming in liquid. In reality, bacteria often live in dense and diverse communities where cell-cell interactions are central to their phenotypes. Different mechanisms of resistance will have a range of consequences for neighbouring bacterial cells, with an impact on their fitness and survival. We have been studying growth in colony biofilms of the opportunistic pathogen *Pseudomonas aeruginosa*. We compete resistant and susceptible strains in varying concentrations of two antibiotics, streptomycin and carbenicillin. These require different resistance mechanisms, carried on the same multi-drug resistant plasmid by the resistant strain in our system. We see susceptible strains can exploit and outcompete resistant strains at some concentrations of carbenicillin. This is explained by cooperative β-lactamase production that protects neighbouring sensitive cells from carbenicillin, by degrading it locally. This is not the case for growth on streptomycin, which is inactivated intracellularly. We also show that this effect depends on the different genotypes being mixed. Our work has further explored these effects with a computational model, considering the possible within-host consequences of these experimental results. In sum, we show that some bacteria locally detoxify their environment of antibiotic and confer protection to nearby susceptible strains. This causes resistance to be selected for at a higher relative sub-inhibitory concentration of antibiotic than in the intracellular case. These effects demonstrate that not all antibiotics are created equal in terms of selection for resistance.

Poster number: 14

Prevalence and optimised detection of resistance to antibiotics in the pig gut microbiome

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Abstract

The use of antibiotics in veterinary medicine, the emergence of antibiotic resistance and the potential transfer of resistance through the food chain to humans are topics of high priority at both the national and EU policy levels as they can negatively impact on animal and human health and welfare. This project aims to establish if multidrug resistance to four critically significant classes of antibiotics; polymyxins (colistin), aminoglycosides, cephlosporins, and carbapenems, is present in food-producing animals such as pigs. These antibiotics represent the different classes of antibiotics that are commonly used to treat illnesses in pigs. As colistin is considered the last line of defence in antibiotics it is crucial to identify the mechanisms leading to the resistance to colistin. Charcoal swabs were used to swab the gut of pigs. The swab was re-suspended in PBS and grown on LB agar. Single colonies were selected onto selective agar plates with the aforementioned antibiotics. Minimum inhibitory concentrations (MIC) were determined using agar dilution tests. Of the 431 isolates that grew, 23 showed imipenem resistance, 48 showed colistin resistance and 10 isolates were resistant to all 5 antibiotics. Of the 23 imipenem resistant isolates, 8 tested positive for metallo-beta lactamases via the EDTA Imipenem test. It is imperative that all sources of antibiotic resistance are identified and controlled in order to minimise the transfer of resistance genes and/or bacteria within animals and between animals and humans.

Poster number: 15

New tricks for old drugs - Revealing the mechanism of action of thioridazine in *Salmonella*

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Abstract

Background. Thioridazine (TZ) is an antipsychotic compound that acts against antibiotic resistant bacteria. However, its mechanism of action has never been fully described. The main aim of this study was to uncover the mechanism of action of TZ, using *Salmonella enterica* serovar Typhimurium as a model bacterium.

Methods. The MIC of TZ was initially determined by broth microdilution method. Analysis of growth kinetic of *S.* Typhimurium 14028S in the presence of several concentrations of TZ was assessed. Motility assays were conducted by exposing *Salmonella* to ½ and ¼ MIC of TZ. Membrane permeability assays were also performed with sub-MIC concentrations of TZ. Adaptation assays were performed by sub-culturing the bacteria during 5 days into new media containing TZ.

Results. The MIC of TZ against *Salmonella* 14028S was 200mg/L. In the presence of TZ and during the first 6-8 hours of exposure to the compound the growth of *Salmonella* was inhibited by sub-MIC. After that, the bacterium grew at a rate similar to that of the control. Decreased bacterial motility was obtained when the bacteria were incubated in the presence of ½ and ¼ MIC of TZ. After the first day of sub-culturing, the bacteria got tolerant to TZ and no inhibition of growth was obtained as in the first 6-8 hours.

Conclusion. The results obtained suggest that TZ may act by targeting the bacterial cell-envelope. When *Salmonella* was exposed to TZ its motility decreased which may be related to the activation of genes involved in drug resistance and envelope stress responses.

Poster number: 16

Repurposing zinc and cobalt organometallic compounds as effective antimicrobials against Gram-positive and -negative bacteria

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Abstract

Background. Due to the rise in antibiotic resistance there is need for new and more effective antimicrobial compounds. The lack in the discovery of novel molecules has turned the attention into repurposing already existing drugs, from which anticancer drugs are one possible source. In this work, we screened the potential antimicrobial activity of two zinc- and one cobalt-organometallic compounds (identified as TS262, TS265 and TS267), previously reported as having anticancer properties.
Methods. The compounds used were tested against *E. coli* ATCC25922, *S.* Typhimurium 14028S and *Staphylococcus aureus* ATCC25923.The determination of the minimum inhibitory and minimum bactericidal concentrations (MIC/MBC) of the compounds, were used to assess their potential antibacterial activity. Toxicity assays were conducted in normal human fibroblasts to determine possible toxicity levels in human cells.
Results. For the three compounds tested the MIC value was equal to the MBC, as follows: *E. coli* (2mg/L), *S*. Typhimurium (2mg/L for TS262 and 4mg/L for TS265 and TS267) and *S. aureus* (1mg/L for TS262 and TS265 and 2mg/L for TS267). In terms of toxicity, the Co(II) coordination compound CoCl(H2O)(phendione)2][BF4] (phendione = 1,10-phenanthroline-5,6-dione) - TS265, demonstrated lower cytotoxicity towards normal human fibroblasts.
Conclusion. These results are promising and further studies on these compounds will hopefully lead to the development of new antimicrobial drugs. Drug repurposing can be an alternative strategy to fight infections caused by antibiotic resistant bacteria.

Poster number: 17

Plasmid mediated colistin resistance encoding genes *mcr-1* and *mcr-2* not detected in *E. coli* isolated in Ireland from retail meats and people

Carina Brehony[1], Blathnaid Mahon[1], Siobhan Kavanagh[1], Martin Cormican[1], Robert Madden[2], Catherine Ludden[1], Carmel Kelly[2], Lynn Moran[2], Cyril Carroll[3], James Bray[4], Keith Jolley[4], Martin Maiden[4], Dearbhaile Morris[1]

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Abstract

Background

The plasmid-mediated colistin resistance gene, *mcr-1*, was first described in November 2015 in *E. coli* isolated from food, animals and humans. Colistin resistance is a major cause for concern as it is one of the very few antimicrobial agents available for treatment of infection associated with carbapenemase producing *Enterobacteriaceae*. Subsequently, *mcr-1* and the related *mcr-2* have been isolated globally from bacteria from various sources. The transmissibility of these genes raises concerns of the rapid and widespread dissemination of colistin resistance.

Methods

Whole genome sequences of 96 *E. coli* isolates collected from retail meats in the island of Ireland (November 2013 - September 2014) and 96 *E.coli* isolates collected (2005-2011) primarily from residents of long term care facilities were examined. Genomes were hosted in and analysis was performed using a BIGSdb *E. coli* database. *mcr-1* and *mcr-2* sequences were used to conduct a BLASTN search against all 192 Irish human and food *E. coli* genomes.

Results

No significant matches were returned indicating the absence of the genes in this set of genomes.

Conclusions

The absence of *mcr-1* and *mcr-2* in this limited collection of food and human genomes from Ireland suggests that it has not yet been disseminated widely in food animals or humans in this region though further testing including retrospective will be required to confirm this. Use of colistin and related compounds in human health care on the island of Ireland is very limited. However, the spread of this transferable colistin resistance mechanism globally is of major concern and underlines the necessity of continuous surveillance.

Poster number: 18

Whole genome sequence analysis of antimicrobial resistant *E. coli* isolated from Irish retail meats

Carina Brehony[1], Blathnaid Mahon[1], Martin Cormican[1], Robert Madden[2], Carmel Kelly[2], Lynn Moran[2], Siobhan Kavanagh[1], Cyril Carroll[3], James Bray[4], Keith Jolley[4], Martin Maiden[4], Dearbhaile Morris[1]

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Abstract

Background

The appropriate and inappropriate use of antimicrobials in human and veterinary medicine, and agriculture, for decades has resulted in the emergence and dissemination of antimicrobial-resistant bacteria. Such antimicrobial resistance is recognised globally as a major public health concern. This study examined the role of food in the dissemination of antimicrobial resistant bacteria in Ireland.

Methods

600 raw meat samples were purchased from retail outlets throughout the island of Ireland Nov 2013 - Sept 2014. All samples were tested for antimicrobial-resistant *E. coli* (AREC) and 496 AREC isolates were obtained. Isolates were characterised by a series of phenotypic and genotypic tests and based on these results, 96 were selected for WGS. Isolate genomes were hosted in and analysis was performed using a BIGSdb database. Core genome MLST (cgMLST) was carried out using an *E. coli* cgMLST scheme downloaded from Enterobase (http://enterobase.warwick.ac.uk/).

Results

54 isolates were extended-spectrum beta-lactamase producers - 33 had blaCTX-M, 19 blaTEM, 18 blaSHV. There were 46 multilocus sequence types, 12 clonal complexes and 61 ribosomal sequence types. In a cgMLST comparison, 2513 loci were compared for all genomes and the fewest differences (<5 loci) were amongst isolates expressing the same antimicrobial resistance phenotype and were temporally and/or geographically related.

Conclusions

AREC found in Irish retail meats were relatively diverse and within that of a globally diverse set of *E. coli* genomes with no distinct separation from human-related isolates. Interrogation of whole genome databases for emerging antimicrobial resistance determinants provided a rapid low cost approach to evaluate the extent of dissemination prior to recognition and will become a more powerful tool as databases expand.

Poster number: 19

Resistance to antimicrobial cidal activity in *Mycobacterium tuberculosis* tested directly from sputum samples reveals distinct effects on the sub-populations present

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Abstract

Sputum samples from patients with tuberculosis contain sub-populations of *Mycobacterium tuberculosis* (Mtb) in multiple physiological states that can be recognised by microscopy, transcript analyses and in vitro cultivation that differentiates colony forming cells from those requiring supplementation in liquid media (differentially culturable cells; DCs) to grow. We examined the cidal effects of anti-tuberculous agents in an assay applied directly to decontaminated sputum samples from patients prior to treatment.

Fresh sputum samples were decontaminated in NaOH and stored in 10% glycerol as previously described (http://dx.doi.org/10.1128/AAC.01380-15). This retains the same balance between the colony forming and DC populations recognised by supplementing with resuscitation promoting factor as that demonstrable in fresh samples and enables multiple exposures to be achieved with the same sample. Exposure to first and second line anti-tuberculous agents over 7 days in 7H9 medium revealed high levels of tolerance to streptomycin and isoniazid in both the colony forming and the DC populations and susceptibility to fluoroquinolones in the latter. These features were completely lost on subculture. With the exception of rifampicin, responses were largely uniform across multiple samples exposed to the same agents.

Our results clearly demonstrate that ex vivo Mtb cells in sputum show very different cidal responses to antimicrobial agents compared to their in vitro propagated counterparts. This presumably reflects that the sub-populations present are in physiological states that are not replicated in vitro. We briefly discuss our attempts to replicate these conditions in vitro and consider the wider implications of these "persister-like" phenomena.

Poster number: 20

Proteomic and virulence analyses of *Staphylococcus Aureus* small colony variants induced by antimicrobial exposure

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Abstract

Backgrounds

Staphylococcus aureus Small Colony Variants (SCVs) are a slow growing subpopulation of bacteria that form in response to environmental stress, such as exposure to antimicrobials. Increased antibiotic susceptibility has been previously reported for SCVs induced by exposure to the antimicrobial compound triclosan although the implications remain unclear.

Objectives

The current study investigates the relative pathogenicity in *S. aureus* SCVs induced by repeated exposure to triclosan and evaluates underlying mechanisms through proteomic analyses

Methods

A selection of *S. aureus* isolates were exposed to triclosan ten times to generate P10 and a further ten times without antimicrobial (PX10). The relative pathogenicity of P10, PX10 and unexposed bacteria (P0) was assessed using wax worm (*Galleria mellonella)* model and using primary human keratinocytes. After triclosan exposure, 4/5 strains formed SCVs. 3/4 SCVs exhibited a significant reduction in relative pathogenicity, evidenced by reduced virulence against *G. mellonella*. Keratinocyte invasion assays showed a reduction in virulence in 2/4 SCVs. Protein analysis revealed overexpression in triclosan target, FabI, and down-regulation in cell adhesion proteins (fibrinogen-binding protein and staphylococcal complement inhibitor) and leukocidin/hemolysin toxin.

Conclusions

Relative pathogenicity was impaired in *S. aureus* SCV induced following triclosan exposure. Whilst it has been suggested that SCVs are associated with enhanced persistence, we have shown impaired keratinocyte invading ability in a section of SCV that were induced by sub-lethal exposure to triclosan.

Poster number: 21

The antibiotic susceptibility of water based nosocomial pathogens *Ralstonia pickettii* and *Ralstonia insidiosa*

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Abstract

Ralstonia pickettii and *Ralstonia insidiosa* are waterborne bacteria that can survive and grow in various water sources that are emerging pathogens in hospital settings. Previous reports on their antimicrobial susceptibility have been largely limited to a few clinical strains with no accounting for genotypic or phenotypic diversity or that these species could vary from the set breakpoints. E-tests and disc diffusion tests were carried out to compare the antimicrobial susceptibilities to twelve different antibiotics of sixty-eight different isolates of *R. pickettii* (fifty-three) and *R. insidiosa* (fifteen) from varying environments, which have previously been well characterised both phenotypically and genetically. The majority of the *R. pickettii* and *R. insidiosa* isolates showed susceptibility to most of the antibiotics tested in this study. The most effective were found to be the quinolones and sulphamethoxazole/trimethoprim. Antibiotic susceptibility was also found not to vary between environmental niches for *R. pickettii* and *R. insidiosa* isolates.

Poster number: 22

Using membrane encapsulated nanoreactors to study antimicrobial resistance mechanisms

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Abstract

This project aims at engineering synthetic self-contained energy-transducing biomembrane vesicles that can serve as nanoreactors, incorporating both natural and bespoke membrane proteins. The ultimate goal is to control the flux of specific molecules into or out of the nanoreactors and the chemistry within them. This will allow us to study antimicrobial resistance mechanisms and aid in identifying new drug targets.

In order to achieve this, a range of component membrane proteins have been produced, which will act as gateways to control the influx and efflux of molecules and ions in a contained phospholipid environment. These include transporters, e.g. AcrB and AmpG, sugar transporters, e.g. SglT, the light-driven sodium pumping rhodopsin NaR (KR2) and the light-driven proton pump – bacteriorhodopsin (bR). Various methods are being developed to control the orientation, stoichiometry and self-assembly of the components upon reconstitution into liposomes.

Moreover, various assays are being explored for the purpose of monitoring membrane transport in assembled nanoreactors using spectroscopic methods, including assays from antibiotic and sugar transport.

This system will be further developed for the purpose of catalysis, synthesis of chemicals and degradation of toxic molecules.

Poster number: 23

Elucidating the role of copper resistance in promoting the survival of *Staphylococcus aureus* in macrophages

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Abstract

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major problem for animal and human health and considered a high priority pathogen by the World Health Organisation. Copper is used agriculturally in animal feed as a growth promoter exerting a selective pressure for bacteria with copper resistance in the environment. Many successful contemporary clones of MRSA carry copper resistance genes on mobile genetic elements such as plasmids. Copper resistance is potentially important for survival in macrophages because macrophages import toxic levels of copper into the phagosome to kill bacteria. Here the contribution of the plasmid-borne copper resistance genes *copB* and *mco* to the intracellular survival of MRSA in macrophages was studied. A MRSA strain carrying the copper resistance plasmid showed hyper-resistance to copper in vitro and significantly higher survival in macrophages compared to the same strain without the plasmid suggesting that factors carried on the plasmid promote resistance to copper and macrophage killing. Bi*nding* of *Cu(II)* to the copper-sensing transcriptional repressor CsoR induces *derepression* of *copper* resistance genes in *S. aureus*. To test if copper resistance was responsible for survival in macrophages, a mutant expressing a variant CsoR protein (CsoR Cys41Ala/His66Ala/Cys70Ala) that cannot coordinate Cu^{2+} and remains bound to the *copB/mco* promoter was tested. This CsoR mutant was less well able to survive in macrophages than the wild-type, presumably because depression of copper resistance genes did not occur. Thus, copper resistance genes may enhance the fitness of MRSA by increasing resistance to the copper-dependent bactericidal activity of macrophages.

Poster number: 24

Framework model for assessing human exposure to antibiotic resistant bacteria through drinking water

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Abstract

Antibiotic resistant (AR) infections lead to thousands of deaths each year worldwide. Research shows the occurrence of AR bacteria (ARB) in various water ecosystems around the world, however little is known about potential human exposure to ARB through drinking water. A framework model is presented which examines the potential human exposure to AR *E. coli* through drinking water. The influence of environmental factors (sunlight, salinity, and water temperature) and drinking water treatments (chlorination, carbon filtration, sand filtration, UV and ozone) on AR *E. coli* is considered in a number of scenarios to estimate likely levels (if any) after treatment. Input uncertainty and variability was modelled using probability distributions and Monte Carlo simulation (10,000 iterations) was used to generate output distributions using the @ risk software. Preliminary results show UV treatment gives the largest reduction of AR *E. coli* out of all the water treatment scenarios examined. However, it is noted exposure to sunlight after this treatment can lead to photoreactivation of UV-damaged bacterial cells. Through a series of scenarios, the model highlights combinations of water treatments that are more favorable at reducing the risk of human exposure to AR *E. coli* through drinking water.

Poster number: 25

Helicobacter pylori Strain Diversity in a Cohort of Adolescents and Their Family Members in a Developed Country

Brendan Dolan[1,2], Lucy Burkitt-Gray[1,2] Stephen Shovelin[1], Billy Bourke[1,2,3], Brendan Drumm[1], Marion Rowland[1] and Marguerite Clyne[1,2]

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Abstract

Background: *Helicobacter pylori* infection occurs mainly within families before the age of 5 but the route of transmission is unknown. The use of stool specimens to genotype strains facilitates the inclusion of large numbers and complete families in transmission studies. We aimed to use molecular genetic analysis of DNA from stool to analyze strain diversity in *H. pylori* infected families. Methods: Using stool specimens we used an antibody capture technique to concentrate the low number of *H. pylori* present in stool specimens prior to DNA isolation and genotyped *H. pylori* strains using specific biprobe qPCR analysis of *glmM, recA* and *hspA* genes. Results: Concentration of *H. pylori* organisms before DNA isolation enhanced subsequent DNA amplification. We isolated *H. pylori* DNA from 50 individuals in 13 families. T_m data for at least 2 of the 3 genes and sequencing of the *glmM* amplicon were analyzed. In line with previous studies similar strains were commonly found in both mothers and children and in siblings. However, 20/50 (40%) individuals had multiple strains and several individuals harbored strains not found in other family members, suggesting that even in developed countries sources of infection outside of the immediate family may exist. Conclusions: Whether infection occurs multiple times or there is one transmission event with several strains is not known but future studies should aim to analyze strains from children much closer to the onset of infection. Our findings have important implications for interpretation of antibiotic sensitivity results and for the design of treatment strategies.

Poster number: 26

New options for the treatment of *Escherichia coli* ST131 infections defined by bioluminometry

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Abstract

Background:
E. coli ST131 has emerged globally as a multi-drug resistant pathogen causing both community and healthcare-acquired infections. Currently available therapies are often ineffective against infections caused by this clonal group, increasing the risk of adverse clinical outcomes. The aim of this study was to evaluate the utility of bioluminescent reporter plasmids for monitoring cell viability in ST131 in response to an unconventional antibiotic combination.

Methods:
The Lux reporter plasmids, pMH1 and pMH2, were constructed. The utility of these plasmids in monitoring cell viability was tested in a number of clinical isolates. Subsequently, the synergistic activity of sub-inhibitory colistin and vancomycin combination treatment against ST131 clinical isolates was assessed by luminometry.

Results:
pMH1 and pMH2 were stably maintained by the prototypic ST131 isolate EC958. In addition, the plasmids imposed no fitness burden. One light unit was equivalent to ~10 bacteria. Luminescence was strongly correlated with cell viability in EC958pMH1 ($r^2 = 1$) and EC958pMH2 ($r^2 = 0.9998$) during exponential growth. Measurable luminescence was conferred on five additional clinical isolates. Combined treatment with sub-inhibitory colistin and vancomycin was found to be significantly more effective than sub-inhibitory colistin singly for all isolates tested.

Conclusion:
In the absence of suitable therapies for treating potentially lethal multi-drug resistant bacterial infections, combination therapies of currently approved drugs with complementary modes of action should be considered.

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