MICROBIOLOGY SOCIETY

# ANNUAL CONFERENCE ONLINE 2021

26–30 April **2021**

## POSTER ABSTRACT BOOK

#Microbio21

**P001**

# Detection of Extended Spectrum Beta-lactamase Gene (CTX-M) among Representative Multidrug-Resistant Gram Negative Bacterial Isolates from Patients with Urinary Tract Infection in Ekiti State, Nigeria.

Ayodele Oluwaseun Ajayi[1], Samuel Ayodeji Osanyinlusi[1], Amos Ojerinde[2], Bryan Ogeneh[2]
[1]Federal University Oye Ekiti, Oye-Ekiti, Nigeria. [2]Federal University Oye Ekiti, Federal University Oye Ekiti, Nigeria

## Abstract

Urinary tract infection is huge public health burden and the emergence of extended spectrum beta lactamase producing bacterial pathogens increases the burden of infectious diseases in Nigeria. This study determined the current prevalence of cephalosporin resistance among Gram-negative bacteria isolated from patients with urinary tract infections between February 2018 and June 2018. Non-repetitive Gram–negative bacteria were recovered from 106 individuals with urinary tract infections who reported at two tertiary healthcare centers in Ekiti-State, Nigeria. A total of 106 bacterial isolates were obtained which included: *Klebsiella pneumoniae* 34 (29.1%), *Klebsiella oxytoca* 17 (16.0%), *Proteus vulgaris* 10 (9.4%), *E. coli* 24 (22.6%), *Proteus mirabilis* 18 (16.9%) and *Pseudomonas aeruginosa* 3 (2.8%). Sixty five of these organisms showed resistance to ceftazidime while 76 organisms showed resistance to ceftriaxone. Forty representative organisms were selected and tested for presence of extended spectrum beta-lactamase (ESBL) genes using primers specific for different ESBL genes. A total of eight (20.0%) organisms carried the *blaCTX-M* gene and other variants of the ESBL genes were not detected. The organisms carrying the *blaCTX-M* gene included *E. coli* 3 (37.5%), *K. pneumoniae* 1(12.5%), *P. mirabilis* 1(12.5%),) and *K. oxytoca* 3(37.5%). The high prevalence of cephalosporin resistant Gram-negative bacteria among patients with UTI is a serious threat to public health and efforts must be intensified to regulate the clinical use of the cephalosporins.

## P002

# Nitrofurantoin-resistant *Escherichia coli* in the UK: genetic determinants, diversity, and undetected occurrences

Yu Wan[1], Ewurabena Mills[1,2], Rhoda C.Y. Leung[1], Ana Vieira[1], Elita Jauneikaite[3,1], Xiangyun Zhi[1], Nicholas Croucher[3,4], Matthew J. Ellington[5,1], Shiranee Sriskandan[1]
[1]NIHR Health Protection Research Unit in Healthcare Associated Infections and Antimicrobial Resistance, Department of Infectious Disease, Imperial College London, London, United Kingdom. [2]Imperial College Healthcare NHS Trust, London, United Kingdom. [3]Department of Infectious Disease Epidemiology, School of Public Health, Imperial College London, London, United Kingdom. [4]MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College London, London, United Kingdom. [5]Antimicrobial Resistance and Healthcare Associated Infections laboratory, National Infections Service, Public Health England, London, United Kingdom

## Abstract

**Background** Antimicrobial resistance in enteric or urinary *E. coli* might predispose invasive *E. coli* infection and bacteraemia. Nitrofurantoin resistance occurs in <6% of UK urinary *E. coli* isolates, however, 2018 national recommendations to prescribe nitrofurantoin for uncomplicated urinary tract infection (UTI) raised concerns for increased prevalence of nitrofurantoin-resistant *E. coli* in the future. Therefore, we investigated mechanisms of nitrofurantoin resistance in UK *E. coli* isolates and assessed their occurrences in a large dataset of *E. coli* genomes.

**Methods** To elucidate chromosomal and acquired genetic determinants of nitrofurantoin resistance in *E. coli*, we analysed whole-genome sequences of nine randomly selected nitrofurantoin-resistant UTI *E. coli* isolates from West London. We then performed targeted analysis of 12,412 *E. coli* genomes collected from across the UK and predicted nitrofurantoin susceptibility from identified genotypes.

**Results** Using comparative genomics, we found known and novel point mutations or insertion sequences (ISs) in chromosomal genes encoding oxygen-insensitive nitroreductases NfsA and NfsB in the nine isolates. Most of these genetic alterations resulted in gene inactivation. We also identified the same kinds of mutations in NfsA, NfsB, and their associated enzyme RibE in a number of 12,412 *E. coli* genomes. We also observed homoplasic mutations in all these proteins. By contrast, multidrug efflux pump OqxAB, which confers resistance when horizontally transferred, was only encoded by one genome.

**Conclusions** Chromosomal *de novo* mutations and ISs are main causes of nitrofurantoin resistance in UK *E. coli*. Prevalence of nitrofurantoin resistance should be monitored among urine, blood, and enteric isolates as nitrofurantoin exposure increases.

## P003

# Trans-cinnamaldehyde - geraniol mixture – antibacterial activity and haemotoxicity

Daria Olkiewicz[1], Maciej Walczak[1], Magdalena Stepczyńska[2]
[1]Nicolaus Copernicus University, Toruń, Poland. [2]Kazimierz Wielki University, Bydgoszcz, Poland

## Abstract

**Abstract**

The aim of this study was to test the antibacterial activity and haemotoxicity of (trans)-cinnamaldehyde and geraniol. Conducted analyzes showed that (trans)-cinnamaldehyde- geraniol mixture have antibacterial properties against *S. aureus* ATCC6538, *E. coli* ATCC8739 and *P. aeruginosa* KKP991. MIC and MBC of the mixture was determined for each strain. For *S. aureus* MIC=0.065 mg/ml, MBC=1.25 mg/ml, for *E. coli* MIC= 0.5 mg/ml, MBC=1 mg/ml and for *P. aeruginosa* MIC=0.5 mg/ml, MBC=1.25 mg/ml. Also the blood compatibility test was done, and it showed that MIC for *S. aureus* (0.025 mg/ml) is non-haemotoxic, but other MICs and all MBCs, unfortunately are.

## P004

# Phytochemicals As Novel Alternative Antimicrobials

Ryan Sweet, Mark Webber, Paul Kroon
Quadram Institute Bioscience, Norwich, United Kingdom

## Abstract

As the incidence of AMR rises globally, and discovery of novel antibiotic classes remains stagnant, novel antimicrobials are urgently required to control AMR pathogens within a wide variety of clinical and non-clinical contexts. Phytochemicals, especially polyphenols, include secondary metabolites produced by plants for numerous functions; one such being as antimicrobial defences. These compounds are a promising source of novel bioactive structures and have been investigated for their potential as alternative sources of antimicrobials.

We screened a phytochemical panel for antimicrobial activity against the common foodborne pathogens *Salmonella enterica*, *Staphylococcus aureus, Listeria monocytogenes* and *Pseudomonas aeruginosa*. A range of phenotypic assays were used to investigate the impact of the polyphenols on growth rates and cellular permeability, as well as to identify compounds that synergised with existing antibiotics. Various phytochemicals exhibited antimicrobial activity; naringenin reduced the growth rate of *Salmonella* by ~30% and *Staphylococcus* by >95%. Thymol was particularly potent reducing the growth rate of *Salmonella* and *Staphylococcus* by ~47% and ~48% respectively, with a >95% reduction in the growth rate of *Pseudomonas*. In terms of synergistic capacities, caffeic acid significantly increased the membrane permeability of *Salmonella* by ~46%, a property which holds promise for potentiating known antibiotic classes in synergistic antimicrobial chemotherapies.

The potential for selection of mutants resistant to thymol was investigated using selective agar-plating methods, and was observed to induce tolerant mutants at a frequency of ~$3.77 \times 10^{-8}$ for *Salmonella, Staphylococcus* and *Pseudomonas*. The data gathered throughout this project highlights the promise of phytochemicals as alternative sources of antimicrobials, with both direct and synergistic capabilities.

## P005

# Antimicrobial resistance Salmonella isolates recovered from food products of animal origin in the Russian Federation

Galina Skitovich, Kseniya Serova, Yevgenia Korchagina, Natalya Shadrova
FGBI "Federal Centre for Animal Health", Vladimir, Russian Federation

## Abstract

The study was aimed at Salmonella isolation from samples of animal food products submitted for testing from various regions of the Central part of the RF and serotyping of the recovered isolates and their testing for antibiotic resistance. A total of 2,342 tests were performed and 87 (3.7%) *Salmonella* isolates were recovered. Most of them (54 isolates) were recovered from poultry meat and poultry meat preparation samples submitted for testing. Besides, 25 isolates were recovered from pork and pork preparation samples, 7 isolates – from beef samples, 1 isolate – from hard cheese samples. Serotyping of 64 *Salmonella* isolates showed that the majority of the isolates (57.8 %) belonged to $O_7$ group. Also, *Salmonella* isolates belonging to $O_9$ (21.9%), $O_8$ (9.4%), $O_{4,5}$ (6.2%) and $O_{10}$ (4.7%) were detected in food products. *S. Enteritidis*, (23.3%), and *S. Infantis* (18.7%), were predominant based on the number of detections. Also, the following serovars were identified: *S. typhimurium*, *S. nigeria, S. montevideo, S. derby, S. meleagridis, S. virchov, S. oranienburg*. Tests of 87 *Salmonella* isolates for their antibiotic resistance with disk diffusion method revealed that they were highly resistant to nalidixic acid (70.1%), tetracycline (49.4%), trimethoprim/sulfamethoxazol (40.2%). Moreover, nalidixic acid-resistance was common for all identified isolates. Seventeen isolates (19.5%) demonstrated multiple antibiotic resistance and two isolates were found to be resistant to ≥7 antibiotics. All recovered isolates were susceptible to gentamicin, amikacin, meropenem and imipenem. Obtained results indicate the necessity of *Salmonella* antibiotic resistance monitoring to gain understanding of *Salmonellas*' antibiotic resistance emergence and trends.

## P006

# The ecology and antimicrobial resistance of Staphylococci colonising neonates

Heather Felgate[1], Dheeraj Sethi[2,1], Kirstin Faust[3], Paul Clarke[4,5], Christoph Härtel[3], Jan Rupp[6], Mark Webber[1,5]
[1]Quadram Institute BioScience, Norwich, United Kingdom. [2]Norfolk and Norwich Hospital, Norwich, United Kingdom. [3]Department of Pediatrics, University of Lübeck, Lübeck, Germany. [4]Neonatal Unit, Norfolk and Norwich University Hospital, Norwich, United Kingdom. [5]Norwich Medical School, University of East Anglia, Norwich, United Kingdom. [6]Department of Infectious Diseases and Microbiology, University of Lübeck, Lübeck, Germany

### Abstract

Coagulase Negative Staphylococci (CoNS) are common commensals of human skin, account for nearly 20% of the microbiota in infants and are thought to promote early immune responses in healthy babies. However, CoNS are opportunistic pathogens and in the UK between 2005 and 2014 were responsible for 57% of episodes of Late Onset Sepsis (LOS). In neonatal intensive care units (NICUs) this is a major concern and antiseptics are used to prevent vascular catheter infections. Chlorhexidine (CHX) and Octenidine (OCT) are the most common agents used for skin antisepsis, but evidence is emerging of antiseptic tolerance amongst CoNS.

We undertook a longitudinal survey of CoNS from skin and rectal swabs isolated from babies in two NICUs from countries with different antiseptic regimens (UK and Germany). Over 1000 isolates were characterised for antimicrobial susceptibility and sequenced. The most frequent species isolated were *S. epidermidis* and *S. haemolyticus* with similar strain types present in both units. Reduced susceptibility to CHX and OCT was observed in UK isolates (where CHX is used), compared to German isolates (where OCT is used).

Analysis of genome data using GWAS and clustering techniques has identified loci associated with antimicrobial susceptibility. Comparison of isolates taken on admission and thereafter, demonstrated that babies acquired isolates with decreased antiseptic tolerance after admission. This data provides new information about the phylogeny of CoNS in NICUs and suggest different potentials for selection of resistance between antiseptics commonly used in neonatal care.

## P008

# Investigating the impact of growth environment on antibiotic resistance: How gold is the golden standard?

Jenny Littler, Freya Harrison, Xavier Didelot
University of Warwick, Coventry, United Kingdom

### Abstract

Antibiotic resistance is of global concern. The growth environment is an important factor when assessing resistance and virulence, diagnostic tests don't often take this into account.

In vitro tests are regularly performed in standard laboratory media: however, it is unclear how accurately these reflect *In vivo* results compared with more clinically realistic models.

We performed a number of tests on *Pseudomonas aeruginosa* LESB58*,* a pathogenic bacterium which infects the lungs of people with CF, in either Mueller Hinton broth (MHB), artificial cystic fibrosis sputum media (ASM) or synthetic wound fluid (SWF).

Virulence assays measuring pyoverdine and pyochelin levels are consistently higher in the more clinically relevant media ASM and SWF compared with MHB. We then assessed the minimum inhibitory concentrations (MICs) in the presence of colistin or meropenem between the different media. MIC results for colistin in ASM - 4mg/ml, MHB - 4mg/ml and SWF – 2mg/ml. MIC results for meropenem in ASM - 4mg/ml, MHB - 8mg/ml and SWF - 16mg/ml. Following this, we tested the concentration of colistin needed to eliminate a biofilm in an *Ex vivo* pig lung, which was 256mg/ml.

These results show the extent to which growth environment is an important condition when looking at *In vitro* diagnostic tests, including the effect of biofilm formation on antibiotic susceptibility.

Future experiments include evolving *P.aeruginosa* in the various media with increasing concentrations of antibiotic to assess any differences in the evolved genetic resistance. Metabolomic profiles of the bacterium grown in the different media will also be assessed.

## P009

# Understanding mechanisms of β-lactam antibiotic resistance in *Streptococcus pneumoniae*

Carolin M. Kobras, Andrew K. Fenton
The Florey Institute for Host-Pathogen Interactions, University of Sheffield, Sheffield, United Kingdom

## Abstract

The human pathogen *Streptococcus pneumoniae* causes many serious invasive infections, including sepsis, meningitis and pneumonia. Combined, these diseases are a major cause of human mortality with the highest infection rates among infants and the elderly. In the clinic, *S. pneumoniae* infections are usually treated with β-lactam antibiotics, but an alarmingly large number of clinical isolates have developed resistance against this treatment approach. To counteract the emergence of resistance, it is important to understand the mechanisms that underpin these phenotypes. This study will identify and characterise novel genetic pathways and cellular processes linked to beta-lactam resistance in *S. pneumoniae*.

High levels of β-lactam resistance in *S. pneumoniae* are often associated with dramatic changes in the penicillin-binding proteins. These changes reduce the bactericidal effect of the antibiotic treatment while maintaining the essential cellular function of these enzymes. However, acquisition of these divergent penicillin-binding proteins is not always sufficient to establish β-lactam resistance and often additional mutations in the genome are required. To identify the genes involved in resistance, we have performed a whole-genome fitness profiling technique (Tn-seq) in the presence of antibiotic selection. Additionally, we have generated strains able to resist β-lactam antibiotics in laboratory conditions, independent of any alterations in their penicillin-binding proteins. Here, we discuss the pathways identified using both techniques, putting them into context with our current understanding of bacterial physiology.

Ultimately, we aim to 're-sensitise' antibiotic resistant *S. pneumoniae* strains by undermining the identified resistance mechanism, in order to safeguard this important treatment approach for future clinical use.

## P010

# Colistin kills bacteria by targeting lipopolysaccharide in the cytoplasmic membrane

Akshay Sabnis[1], Katheryn Hagart[1], Anna Klöckner[1], Michele Becce[1], Lindsay Evans[2], Chris Furniss[3], Despoina Mavridou[4], Ronan Murphy[5], Molly Stevens[1], Jane Davies[5], Gerald Larrouy-Maumus[1], Tom Clarke[1], Andrew Edwards[1]
[1]Imperial College London, London, United Kingdom. [2]The Institute of Cancer Research, London, United Kingdom. [3]Stockholm University, Stockholm, Sweden. [4]The University of Texas at Austin, Texas, USA. [5]National Heart and Lung Institute, London, United Kingdom

## Abstract

Colistin is an antibiotic of last resort, but has poor efficacy and resistance is a growing problem. Whilst it is well established that colistin disrupts the bacterial outer membrane by selectively targeting lipopolysaccharide (LPS), it was unclear how this led to bacterial killing. We discovered that MCR-1 mediated colistin resistance is due to modified LPS at the cytoplasmic rather than outer membrane. In doing so, we also demonstrated that colistin exerts bactericidal activity by targeting LPS in the cytoplasmic membrane. We then exploited this information to devise a new therapeutic approach. Using the LPS transport inhibitor murepavadin, we were able to cause LPS accumulation in the cytoplasmic membrane, which resulted in increased susceptibility to colistin *in vitro* and improved treatment efficacy *in vivo*. These findings reveal new insight into the mechanism by which colistin kills bacteria, providing the foundations for novel approaches to enhance therapeutic outcomes.

## P011

# Interspecies interactions provide antibiotic protection within cystic fibrosis bacterial communities

Michael Bottery, Jon Pitchford, Ville Friman
University of York, York, United Kingdom

## Abstract

Bacteria have social lives—they are able to signal with each other to coordinate communal activities. These social traits are critical for the growth and survival of bacteria in harsh environments. Many of these social interactions are intended for cooperation between members of the same strain or species, but the benefits of some cooperative traits are leaky and can be exploited by different species within a bacterial community. Hence, the ecological context of a pathogen could alter our ability to treat infection. We show that interspecies interactions during polymicrobial infections of the Cystic Fibrosis lung can provide focal pathogens with antibiotic protection. Using model CF lung communities, we demonstrate that the focal pathogen *Pseudomonas aeruginosa* can socially exploit antibiotic resistance in the presence of *Stenotrophomonas maltophilia,* a bacterium that can hydrolyse imipenem antibiotic. The ecology of the community is key to this protection, as the extent of protection provided is dependent upon the density of *S. maltophilia* and is not provide by the presence of other commonly co-occurring CF pathogens, such as *Staphylococcus aureus*. These findings reveal that social exploitation of pre-occurring antimicrobial resistance, and inter-specific competition, can have a large effect on the efficacy of antibiotic treatments highlighting the importance of microbial ecology for understanding the efficacy of antibiotic treatments.

## P012

# Enzyme-Degradable Polyion-Complex (PIC) Particles for the delivery of antimicrobial peptide polymyxin B.

Sameh El Sayed, Paco Fernandez-Trillo
School of chemistry, University of Birmingham, Birmingham, United Kingdom

## Abstract

PIC particles are stabilised by these electrostatic interactions between its components, and thus are especially suited for the delivery of charged (bio)molecules (e.g. nucleic acids and proteins), which are prevalent in nature. This way, delivery vehicles can be formulated without the need to introduce chemical modifications to these (bio)molecules and as a result, the biological activity of these molecules should be maintained upon release.

Herein, we describe novel polyion complex (PIC) particles for the delivery of Polymyxin B (*Pol-B*), an antimicrobial peptide currently used in the clinic as a last resort antibiotic against multidrug-resistant gram-negative bacteria. Towards this end, we have prepared polymer containing peptide sequence (-Glu-Gly-Leu-Ala-) this sequence is selectively degraded by *pseudospin*, an elastase produced by the opportunistic pathogen *Pseudomonas aeruginosa.*[1] A range of conditions for the controlled assembly of Pol-B with a polymer containing peptides has been identified which let us prepare stable colloidal PIC particles containing different Pol-B: Polymer ratios. Their stability under simulated physiological conditions (i.e. pH, osmotic pressure and temperature) characterised. Furthermore, preliminary evaluation of the antimicrobial activity of these Pol-B containing PIC particles has been performed, by monitoring their effect on the growth of *Pseudomonas aeruginosa*, an opportunistic gram-negative bacterium.

- 1) Insua, I., Wilkinson, A. & Fernandez-Trillo, F. Polyion complex (PIC) particles: Preparation and biomedical applications. *Eur. Polym. J.* 81, 198–215 (2016)

**P013**

# Anti-biofilm activity of 1,000-year-old-remedy requires the combination of multiple ingredients.

Jessica Furner-Pardoe[1], Blessing Anonye[1,2], Ricky Cain[1,3], Catharine Ortori[4], Christina Lee[1], David Barrett[4], Christophe Corre[1], Freya Harrison[1]
[1]University of Warwick, Coventry, United Kingdom. [2]University of Central Lancashire, Preston, United Kingdom. [3]Evotec Ltd, Oxford, United Kingdom. [4]University of Nottingham, Nottingham, United Kingdom

## Abstract

Combatting the rise in antibiotic resistance is one of the major challenges in modern science. Studying historical medical remedies could help reveal new antibiotics. Historical medical manuscripts prescribe complex preparations of several ingredients to treat infections, and it is suspected their efficacy may rely on creating a cocktail of natural products. A reconstructed 1000-year-old remedy, containing onion, garlic, wine, and bile salts, was previously shown to kill methicillin-resistant *Staphylococcus aureus*in a mouse chronic wound model, and *Pseudomonas aeruginosa*in an ex vivo chronic lung infection model. Recently, we have shown this activity extends to biofilms of various ESKAPE pathogens, including MRSA and *Acinetobacter baumannii*. Traditionally, natural product research has focussed on isolating active compounds using planktonic cultures, however, here we present data that by doing so we may overlook efficacious mixtures. We present new observations on the activity of the preparation and demonstrate the potent activity against planktonic cultures is achieved with garlic alone. Chemical analysis has identified the compound responsible to be a sulphur compound within garlic (allicin). However, the garlic alone has no activity against biofilms of the same bacteria. In fact, all four ingredients were necessary to get full potent antibiofilm activity. This highlights the importance of interactions within plant mixtures. By incorporating biofilm studies earlier in the search of active compounds, we may highlight potent interactions and generate promising mixtures for the treatment of infections.

## P013

# A stress-full guide to how biofilms evolve antimicrobial tolerance and impacts on other characteristics

Eleftheria Trampari[1], Emma Holden[1], Gregory Wickham[1], Chuanzhen Zhang[1,2], Mark Webber[1,3]
[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]South China Agricultural University, Guangzhou, China. [3]University of East Anglia, Norwich, United Kingdom

### Abstract

Biofilms are inherently tolerant to antimicrobials although the mechanisms that underpin this are not fully understood and it remains unclear how bacteria in a biofilm will evolve in response to antimicrobial stress. We used an experimental evolution model to study responses of *Salmonella* to sub-lethal exposures to clinically-relevant antibiotics. We found that biofilms adapt to low level antibiotic stress and rapidly select fitter mutants under the conditions tested. Analysis of mutants selected showed there was no shared mechanism for adaptation, instead biofilms responded in a drug-specific manner, employing distinct mechanisms of resistance in each case. We identified and characterised novel mechanisms that involved alterations of membrane structures as well as fine tuning of efflux pump activity. Interestingly, in some cases sub-lethal exposure to antibiotics selected for heterogeneity in populations with distinct sub-populations contributing drug resistance, or ability to produce biomass respectively; benefiting the population as a whole. We believe that experimental evolution is a valuable tool to study antimicrobial resistance in biofilms, can accurately predict the emergence of mutations in the real world, and dissect population dynamics within complex microbial communities.

## P014

# Host environment induces daptomycin tolerance in *Staphylococcus aureus*

Elizabeth Ledger[1], Nathalie Reichmann[1,2], Angelika Grundling[1], Andrew Edwards[1]
[1]Imperial College London, London, United Kingdom. [2]University of Oxford, Oxford, United Kingdom

## Abstract

Daptomycin is a last-resort antibiotic for the treatment of invasive diseases caused by methicillin-resistant *Staphylococcus aureus.* However, despite potent activity *in vitro,* daptomycin fails to resolve up to 30% of cases of staphylococcal bacteraemia, suggesting that the host environment reduces bacterial susceptibility to the antibiotic. Using human serum as a model of bacteraemia, we demonstrated that the host environment induced daptomycin tolerance via activation of the GraRS two-component system. Testing of various antimicrobial peptides present in serum revealed that the human cathelicidin LL-37 was able to bind GraS, induce GraRS signalling and confer daptomycin tolerance. Activation of GraRS by serum led to daptomycin tolerance via changes in the staphylococcal cell envelope. For example, GraRS-dependent increases in positive surface charge and peptidoglycan content occurred in serum, leading to reduced daptomycin binding. Additionally, incubation in serum led to a Cls2-dependent increase in the cardiolipin content of the membrane which contributed to daptomycin tolerance. Finally, inhibition of both peptidoglycan and cardiolipin synthesis together completely abolished acquisition of daptomycin tolerance in serum, demonstrating that these processes fully explain the tolerance phenotype. In summary, host LL-37 activates staphylococcal GraRS signalling, causing changes in the cell surface which confer daptomycin tolerance. This demonstrates how host defences can compromise antibiotic efficacy, and also provides a rationale for combination therapies to prevent the development of daptomycin tolerance and reduce rates of treatment failure.

## P015

# Evaluation of a monoglyceride for antimicrobial activity against Gram-positive and -negative bacteria.

Faith Ukachukwu, Raid Alany, Lori Snyder
Kingston University, London, United Kingdom

## Abstract

Background

The Gram-positive bacterium *Staphylococcus aureus*, and Gram-negative *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa* bacteria, have developed multi-drug resistance to currently recommended antibiotic treatment resulting in worse clinical outcomes and significant economic burden. *N. gonorrhoeae* causes the sexually transmitted infection gonorrhoea and *S. aureus* and *P. aeruginosa* causes nosocomial infections including a plethora of infections that affect the skin, lungs, heart, eyes, ears, bones, and blood. Novel therapies are desperately needed to replenish the clinical pipeline as current antibiotic treatments fail.

Methods

Monolaurin was investigated against strains of *N. gonorrhoeae*, *S. aureus* and *P. aeruginosa*. The minimum inhibitory concentration of monolaurin was determined by agar diffusion assay for *N. gonorrhoeae* and broth microdilution assay for *S. aureus* and *P. aeruginosa*. Bactericidal activity of monolaurin was assessed in log reduction assay and the time - kill activity of various concentrations of the monoglyceride was evaluated over 5 hours. At least 4 $\log_{10}$ killing activity was considered bactericidal.

Result

Monolaurin inhibited growth of *S. aureus*, *N. gonorrhoeae*, and *P. aeruginosa* at a range of 0.1mM - 3.13mM concentrations. At 1.5mM concentration, monolaurin killed *S. aureus* and *N. gonorrhoeae* at 180 minutes and 60 minutes, respectively. Monolaurin at 3mM concentration rapidly killed *N. gonorrhoeae* within 2 minutes while 25mM concentration killed *P. aeruginosa* within 60 minutes.

Conclusion

The evidence suggests that monolaurin actively killed Gram-positive and remarkably Gram-negative bacteria. This monoglyceride can potentially be developed as an antimicrobial therapy against these bacterial infections and may prove useful when coinfection with *P. aeruginosa* and *S. aureus* are present.

## P016

# Characterisation of chromosomal and plasmid diversity of carbapenemase-producing *Klebsiella pneumoniae* clinical isolates from a UK hospital

Sarah J Element, Robert A Moran, Willem van Schaik, Michelle MC Buckner
Institute of Microbiology and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom

## Abstract

The number of carbapenem-resistant *Klebsiella pneumoniae* infections is increasing. Carbapenemase genes are often carried on conjugative plasmids and transferred horizontally. Conjugation requires cell-cell contact, and may be more likely in a biofilm due to close cell proximity in this lifestyle. Five carbapenem-resistant *K. pneumoniae* clinical isolates obtained from Birmingham, UK were selected for sequencing and characterisation. A core genome phylogeny revealed that two of five isolates were closely-related (Sequence Type 147). Nucleotide identity between the ST147 isolates was 100% across the whole genome (98% coverage) (BLASTn). Three of five isolates were dispersed among *K. pneumoniae* (ST512, ST14, ST395). Genome assemblies were optimised to circularise contigs and facilitate assessment of plasmids. Plasmid content was almost identical for the two ST147 isolates, with one isolate containing an additional plasmid. Antimicrobial resistance genes were identified along with likely conjugation modules. All isolates contained F-type plasmids. Three of five carbapenemase plasmids appeared to be conjugative based on the sequencing data, and one of five appeared likely to be mobilisable. The ST147 isolates also contained *bla*OXA-181 on the chromosome. Comparisons to sequences in the NCBI GenBank database provided evidence of international dissemination of these carbapenemase-carrying plasmid groups. Future work will determine whether the identified plasmids can transfer by conjugation in planktonic and biofilm lifestyles and assess genotype-phenotype links.

## P017

# Modelling the effects of livestock antibiotic usage on human food-borne disease

Alexander Morgan, Mark Woolhouse, Bram van Bunnik
University of Edinburgh, Edinburgh, United Kingdom

### Abstract

Excessive livestock antibiotic usage has been proposed as a major driver of antimicrobial resistance in human populations. This has led to antibiotic stewardship programs which aim to curtail usage of livestock antibiotics through a "one-health" approach. However, the human health consequences of livestock antibiotic curtailment are poorly understood. In particular, the potential for increases in the carriage of foodborne pathogens in livestock, due to a loss of antibiotic pressure, and subsequent increases in human foodborne disease. Here we use a mathematical model to explore the impact of curtailing livestock antibiotic usage on both antibiotic-sensitive and antibiotic-resistant food-borne disease in humans.

The model identified increases in overall human foodborne disease and a decrease in resistance following livestock antibiotic curtailment. However, this can be mitigated through interventions to reduce animal-to-human transmission, and to a lesser extent, animal-to-animal transmission and the background rate of contamination in livestock. The magnitude of interventions needed to mitigate increases in human foodborne disease was found to vary across different case studies, suggesting that a "one-size fits all approach" across different agricultural settings, livestock hosts and drug/bug combinations will likely not be successful. This work provides an illustrative example of one of the potential consequences of antibiotic withdrawal and how agricultural biosecurity interventions can be employed to mitigate potential negative human health consequences following livestock antibiotic stewardship programs.

## P018

# Potential Antimicrobial Substances from the CharacterizedBioactive Compounds Extracted from Secondary Metabolites of *Aspergillus terreus.*

Afolake Olanbiwoninu
Ajayi Crowther University, Oyo, Nigeria

## Abstract

**Background and Objective:** The menace of MDR bacteria and search for the potent antimicrobial substance remain relevant research. Fungi are commonly recognized as microorganisms that play role in the production of secondary metabolites which are antimicrobials. Therefore, this study focused on the antimicrobial properties of the fungal secondary metabolites and their bioactive compounds. **Materials and Methods:** Soil samples were collected from the rhizosphere region of different farm gardens in Oyo town, Nigeria. Isolation of the fungi was carried out using Potato Dextrose agar, identified through the amplification of the ITS region of the ribosomal RNA operon, and identified to be *Aspergillus terreus*. The isolates were screened for the production of secondary metabolites by batch culture using an incubator shaker at 27°C for 5 days. The metabolite was extracted and concentrated using a rotary evaporator and aliquots of the metabolites were stored at 4°C. Agar-well diffusion assay was employed to evaluate the antibacterial properties of the secondary metabolites of the fungus. The bioactive chemical compounds of the metabolite extracted from fungus were evaluated using the GC-MS technique. **Results:** The bacterial pathogens investigated in this work were multi-drug resistant bacteria with a minimum resistant rate of 61%. The MDR bacterial pathogens were all susceptible to the secondary metabolite of *Aspergillus terreus* in this study. **Conclusion:** The bioactive compounds evaluated in this work showed the occurrence of organic compounds in the metabolite therefore secondary metabolite of *Aspergillus terreus* holds the chance of discovering the novel and potent drug

## P019

# *Streptomyces venezuelae* peptidases with a putative role in resistance to peptide antibiotics

Frank Eardley[1], Eshwar Mahenthiralingam[2], Mark Buttner[3], Brian Jones[1], Susanne Gebhard[1]
[1]University of Bath, Bath, United Kingdom. [2]Cardiff University, Cardiff, United Kingdom. [3]John Innes Centre, Norwich, United Kingdom

## Abstract

Antimicrobial peptides (AMPs) are a promising source of antibiotics, potentially effective against multi-drug resistant bacteria. Whilst AMP resistance among low GC-content Gram-positive bacteria has been studied in detail, the situation in high-GC Gram-positives is poorly characterised. Here, we identify that *Streptomyces venezuelae* is highly resistant to the AMP bacitracin with an MIC of approximately 64 µg/ml, compared to 0.5-4.0 µg/ml in other *Streptomyces* species. Transcriptome analysis of *S. venezuelae* found a large number of putative peptidase-encoding genes strongly up-regulated under bacitracin challenge, leading us to propose that AMP degradation may contribute to the observed resistance phenotype. Indeed, we could show that cell suspensions of pre-induced *S. venezuelae* rapidly reduced the bioactivity of bacitracin at a rate of approximately 12 ng/min/$OD_{600nm}$. To identify the nature of the peptidase responsible for bacitracin degradation, peptidases within the transcriptome dataset were classified using the MEROPS database. These peptidases were then screened for signal sequences using SignalP to find candidates for the extracellular degradative activity observed. A total of 22 extracellular bacitracin upregulated peptidases were identified. The sequence homology of these peptidases across 98 *Streptomyces* species was examined and compared with experimental bacitracin resistance data from 10 *Streptomyces* species. These analyses showed that the abundance of S12 peptidases was a strong predictor of resistance. S12 peptidases are therefore the most likely candidates for degradation-mediated bacitracin resistance in *S. venezuelae.* In addition, we propose that degradation of AMPs may be a widespread mechanism of resistance in the streptomycetes, with potential implication for microbial community interactions.

## P020

# Characterization of a Multidrug-Resistant *Citrobacter pasteurii* isolate carrying $bla_{KPC-2}$ from municipal wastewater.

Michael P Ryan[1,2], Shannon Slattery[1], J Tony Pembroke[1,3]
[1]Department of Chemical Sciences, School of Natural Sciences, University of Limerick, Limerick, Ireland. [2]Applied Science Department, Limerick Institute of Technology, Limerick, Ireland. [3]Bernal Institute, University of Limerick, Limerick, Ireland

## Abstract

A carbapenem-resistant strain, UL-CPE-01, was isolated from a domestic wastewater treatment plant and subjected to antibiotic susceptibility testing showing resistance to a wide range of antibiotics. Complete genome and bioinformatic analysis of UL-CPE-01 was performed to investigate the nature of its resistance determinants and the strain identified as *Citrobacter pasteurii* based on ANI score. *Citrobacter* spp. are part of the normal human and animal intestinal flora and the UL-CPE-01 *Citrobacter pasteurii* strain detected here is closely related to *Citrobacter freundii*, which is an emerging opportunistic nosocomial pathogen. UL-CPE-01 showed both phenotypic and genotypic multiple resistance to aminoglycoside, β-lactams, sulfonamides, fluoroquinolone, rifampicin, phenicol and macrolide antibiotics. The UL-CPE-01 genome revealed 16 acquired antimicrobial resistance gene (ARGs) conferring resistance to eight different antibiotic groups: *dfrA12* (trimethoprim); *sul1* (sulfonamide); *mph*(A), *mph*(E) and *msr*(E) (macrolide); *qnrB4* and *aac(6')-Ib-cr* (fluoroquinolone); $bla_{DHA-1}$, $bla_{OXA-1}$, $bla_{CMY-77}$, $bla_{OXA-10}$, $bla_{TEM-1B}$, $bla_{KPC-2}$ (β-lactams); *arr-3* (rifampicin); *catB3* and *catA1* (chloramphenicol) and *aac(6')-Ib-cr*, *aadA2* and *ant(2'')-Ia* (aminoglycoside). The novel $bla_{KPC-2}$ determinant was found to be associated with in a Tn*4401*-like element.

## P021

# Understanding the causes of persister cell frequency variation in growth curve time series

Saran Davies, Melanie Ghoul, Ashleigh Griffin
University of Oxford, Oxford, United Kingdom

## Abstract

Bacterial persister cells are antibiotic-tolerant and can survive antibiotic doses several times the minimum inhibitory concentration, despite being genetically identical to susceptible cells. Previous research has indicated that persistence is associated with reduced/halted cell division and lower metabolic activity. Therefore, it should be expected that a small subset of the population (typically 0.001-1%) will enter the persistence state when cell division is slower, i.e. during late exponential and stationary phase. To investigate this information, we grew a lab strain of *Pseudomonas aeruginosa* for 48 hours and exposed the cells to antibiotics at different stages of its growth curve. We also used antibiotics from three different classes, with different modes of action; ciprofloxacin, tobramycin and meropenem, to investigate whether the stressor induces the same level of persistence. We found that contrary to previous research, the greatest number of persisters peaked during early exponential growth, declining into stationary phase and persister cell frequency varied significantly depending on the antibiotic used. Our results indicate the necessity to consider persistence on a case by case basis, allowing for differences among growth phase and antibiotic stressors.

## P022

# Mobile antimicrobial resistance in *Neisseria gonorrhoeae*

Ana Cehovin[1], Keith A Jolley[2], Martin CJ Maiden[2], Odile B Harrison[2], Christoph M Tang[1]
[1]University of Oxford, Sir William Dunn School of Pathology, Oxford, United Kingdom. [2]University of Oxford, Department of Zoology, Oxford, United Kingdom

## Abstract

*Neisseria gonorrhoeae* (the gonococcus) is the causative agent of the sexually-transmitted infection gonorrhoea, and has developed resistance to all classes of antimicrobials. In gonococci, plasmids can mediate high-level antimicrobial resistance (AMR) to tetracyclines and ß-lactams. Plasmids can spread through bacterial populations by transformation and conjugation, resulting in the rapid dissemination of traits. Characterisation of plasmids, including understanding their distribution in bacterial populations, is therefore key to understanding bacterial evolution, and in particular the spread of AMR. *N. gonorrhoeae* can harbour three plasmids, conjugative (pConj), ß-lactamase (p*bla*) and cryptic (pCryp). Using genomic and phylogenetic analyses, we show that plasmids are widespread in a large collection of gonococcal isolates from 56 countries. We found that variants of pConj (which can mediate tetracycline resistance) and p*bla* expressing TEM-135 ß-lactamase are associated with distinct gonococcal lineages. Furthermore, AMR plasmids are significantly more prevalent in gonococci from less wealthy countries. Over 94% of gonococci possess the cryptic plasmid (pCryp), and its absence can be correlated with the presence of a novel chromosomal Type IV secretion system. Our results reveal the extent of plasmid-mediated AMR in the gonococcus, particularly in less wealthy countries, where diagnostic and therapeutic options can be limited, and highlight the risk of their global spread.

## P023

# A bioengineered drug delivery system to fight antibacterial resistance

Anna Klöckner[1,2,3], Renee Tonkin[1,2], Adrian Najer[1,2], Cécile Echalier[1,2,4], Mike Potter[1,2], Andrew M. Edwards[3], Molly M. Stevens[1,2,5]
[1]Department of Materials, Imperial College London, London, United Kingdom. [2]Department of Bioengineering, Imperial College London, London, United Kingdom. [3]MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, United Kingdom. [4]Hybrid Technology Hub-Centre of Excellence, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway. [5]Institute of Biomedical Engineering, Imperial College London, London, United Kingdom

### Abstract

Antibiotic resistance and the lack of new antibiotics in development is a growing healthcare emergency. As a consequence, novel treatment strategies are desperately needed and bioengineered drug delivery systems offer a promising approach to bypass antibiotic limitations. The delivery of encapsulated antibiotics has important advantages over standard delivery, such as improved bioactivity, enhanced permeability, decreased cytotoxicity and triggered drug release. Here, we employed multilayered "smart" microparticles as a dual antimicrobial delivery system towards tackling Gram-positive bacteria.

The engineered particles were characterised by nanoparticle tracking analysis (NTA), dynamic light scattering (DLS) and microscopy. Particles carrying fluorophores were synthesised to study the drug release profile upon a biological trigger. Next, particles were loaded with two different types of antimicrobial compounds. Encapsulation efficiency was analysed by High-performance liquid chromatography (HPLC). Finally, loaded particle-bacteria interactions were tested as well as antibacterial activity using fluorescence microscopy, flow cytometry and CFU counting.

Characterising the microparticles confirmed a multilayer structure with a positive charge which facilitated a strong interaction with negatively charged bacteria. Flow cytometry and microscopy revealed specific interactions of bacteria and particles. Additionally, drug release was observed only in the presence of pathogenic bacteria. A loading efficiency between 3-9% was achieved depending on the antimicrobial compounds that were encapsulated. Bacterial time killing assays showed potent antibacterial activity of both the single and dual loaded particles towards *Staphylococcus aureus*.

Overall, the data generated in this project demonstrated the potential of bioengineered multilayered particles as an antibiotic delivery system to fight multidrug resistant bacterial infections.

## P024

# Antimicrobial cationic polymers possess promising antibacterial and anti-biofilm activities as a new class of antibiotics

Ramon Garcia Maset[1], Alexia Hapeshi[1], Stephen Hall[2], Freya Harrison[1], Sebastien Perrier[1]
[1]University of Warwick, Coventry, United Kingdom. [2]ISIS, Didcot, United Kingdom

## Abstract

**Background:**

The rational design of new antibiotic agents is of imminent global necessity as more than 10 million deaths and an immense cost in health systems are prediced by 2050 due to multidrug bacterial infections.

Inspired by natural antimicrobial peptides, synthetic cationic polymers have been recently investigated for their antibiotic potential.

**Materials/methods:**

Cationic polymers mimicking antimicrobial peptides were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization with various architectures and cationic charge types. The positively charged moieties (guanidinium and ammonium) were used to mimic arginine and lysine amino-acids respectively.

Standard antimicrobial susceptibility tests were performed on two strains of *Staphylococcus aureus*.

Mammalial cell proliferation/activity was tested using the XTT assay. Haemolytic activity of the compounds was tested in sheep red blood cells (RBC).

The antibiofilm activity was evalulated using a synthetic collagen wound biofilm model.

**Results:**

The polymers showed good antimicrobial activity against *S. aureus* strains in Muller-Hilton broth (MHB) and in synthetic wound media (SWM) which is more representative of a clinical wound infection environment. The compounds were non haemolytic. However, the polymers and especially guanidinium compounds, showed some toxicity against 3T3 fibroblast cells.

The activity against *S. aureus* biofilms was tested in a collagen wound model. A mature biofilm was formed in the collagen-wound model and afterwards treated with a polymeric solution. A significant decrease in colony forming units (CFU) in the treated wound could be observed.

**Conclusions:**

Antimicrobial cationic polymers possess promising antibacterial and anti-biofilm activities and could form the basis of a new class of antibiotics.

## P025

# A Tale of Two-Component Systems: Dissecting an Antibiotic Resistance Network in *Enterococcus faecalis*

Sali Morris[1], Tim Rogers[2], Susanne Gebhard[1]
[1]Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom. [2]Department of Mathematical Sciences, University of Bath, Bath, United Kingdom

### Abstract

Responsible for an estimated 54,500 hospitalisations and 5400 deaths per year in the US, *Enterococcus* infections have become a significant public health burden, with the emergence of daptomycin (DAP) resistance causing major concern. For *E. faecalis,* the problem is amplified by a remarkable level of innate resistance to cell-envelope acting antibiotics and we propose that this high level of resistance stems from the complexity of *E. faecalis'* Cell Envelope Stress Response (CESR), which remains poorly understood.

Our work has recently unveiled a complex bacitracin resistance network, comprised of the sensory ABC transporter SapAB, the two-component system (TCS) SapRS and the target resistance regulon. Interestingly, components of this system have recently been implicated in DAP resistance, a role usually fulfilled by a second TCS, LiaFSR. We therefore aimed to unravel the interplay between these two regulatory pathways and the coordinated expression of their target resistance genes.

Utilising transcriptional reporter gene fusions and gene deletion strains, we show that LiaFSR regulates the expression of both SapAB and SapRS. This places SapRS target genes under dual control by not only the Sap, but also the Lia regulatory systems. Our results show that this strategy effectively implements a logic 'AND' gate, requiring both antibiotic-induced cellular damage (LiaFSR) and the presence of a substrate drug for the network's sensory transporters (SapAB).

As we begin to shed light on the complexity of this antibiotic stress system, we move closer to identifying an Achilles' heel within the network, which may ultimately lead to the discovery of new therapeutic targets.

## P028

# *In silico* Approaches for Targeting an Essential Mycobacterial Protein, PrsA

Alexander Kingdon, Luke Alderwick
University of Birmingham, Birmingham, United Kingdom

## Abstract

*Mycobacterium tuberculosis* is a leading cause of death worldwide and with increasing cases of drug-resistance, new treatments options are required. PrsA is an essential protein in mycobacteria and is necessary for arabinogalactan production and nucleotide biosynthesis. This protein is not currently targeted by any drugs in clinical development and represents a potential drug development avenue.

Currently, no crystal structure of *M. tuberculosis* PrsA (MtPrsA) is available, hence the SWISS-MODEL structure, based on *M. smegmatis* (93% similarity) was utilised. Molecular Dynamic simulations were performed on the modelled structure, to gain information on the protein's predicted flexibility. The output trajectory was clustered, and representatives of each major cluster were used in downstream analysis. Ensemble docking, molecular docking on the ensemble of MtPrsA structures, of the GSK-177 prioritised compounds was undertaken. The docking predictions were also re-evaluated using machine learning based programmes, including NNScore.

This approach yielded several compounds which could be taken forward for further computational analysis. The compound rankings were also retroactively compared to experimental data studying the GSK-177 compounds' interactions with mycobacteria.

This research represents a potential workflow for future *in silico* drug development efforts against *M. tuberculosis* and further work may allow the identified compounds to be used for the treatment of TB.

## P029

# Characterisation of antibiotic resistant strains of Campylobacter jejuni isolated from humans andchickens

Elizabeth O'Gorman[1], Marguerite Clyne[2], Gillian Carney[1]
[1]UCD School of Biomolecular and Biomedical Science, Dublin, Ireland. [2]UCD School of Medicine, Dublin, Ireland

## Abstract

Campylobacter jejuni is a common foodborne pathogen that is becoming increasingly resistant to fluoroquinolone and tetracycline antibiotics. Antibiotic resistance can provide selective advantages for bacteria in the absence of antibiotics. There is a need to identify common characteristics of antibiotic resistant Campylobacter in order to identify strains that pose a significant risk to human health. This study aimed to identify phenotypes associated with ciprofloxacin and tetracycline resistance from strains isolated from humans and from chickens. Forty strains of C. jejuni were tested for resistance to ciprofloxacin and tetracycline. Twenty-nine strains (73%) were resistant to both antibiotics. Only eight strains were resistant to just one antibiotic. Resistant strains were isolated in nearly equal numbers from both sources. PCR screening lead to the identification of a mosaic tetracycline gene tetO/32/O-ii which provides a minimum inhibitory concentration (MIC) for tetracycline of 64mg/L or higher. Antibiotic resistance was not shown to influence motility or growth levels at different temperatures, but the origin of the strain did. Chicken isolates had higher motility levels, grew better at 42℃, and had higher survival rates at 4℃. From this study, it can be concluded that high levels of antibiotic resistance occur in C. jejuni with fluoroquinolone and tetracycline resistance commonly occurring together. Future studies should investigate how antibiotic resistance affects phenotypes in the presence and absence of antibiotic use.

## P030

# Does diclofenac select for antimicrobial resistance genes in a complex microbial community?

April Hayes[1], Lihong Zhang[1], Jason Snape[2], William Gaze[1], Aimee Murray[1]
[1]University of Exeter, Penryn, United Kingdom. [2]AstraZeneca, Macclesfield, United Kingdom

## Abstract

The non-antibiotic drug diclofenac has been demonstrated to have antimicrobial effects on bacterial strains, often at high concentrations. However, this analgesic is also frequently found in the freshwater environment worldwide, at much lower concentrations. Previously, we demonstrated diclofenac had an effect on the growth of a complex bacterial community derived from wastewater influent, using a method that shows growth reduction can be a proxy for selection for antibiotic resistance genes. Here, using metagenomics, we confirm whether diclofenac can co-select for antimicrobial resistance genes within the same bacterial community. We evolved a wastewater community under exposure to a range of diclofenac concentrations, from human gut concentrations to those found in the freshwater environment. Our findings will help address the knowledge gap surrounding whether non-antibiotic drugs can co-select for antimicrobial resistance genes in human gut and environmental microbiomes.

## P031

# Colonisation by multi-drug resistant *Escherichia coli* in the absence of antibiotic selection

Chris Connor[1], Amanda Zucoloto[2], Braedon McDonald[2], Alan McNally[1]
[1]University of Birmingham, Birmingham, United Kingdom. [2]University of Calgary, Calgary, Canada

## Abstract

Antimicrobial resistance (AMR) is a growing health concern with pathogenic bacteria becoming increasingly resistant to clinically used antibiotics. *E. coli* account for a significant proportion of these multi-drug resistant (MDR) infections. Of particular note is *E. coli* ST131 which has established itself as a globally dominant MDR clone. Traveller surveillance studies have indicated that MDR bacteria are capable of colonising healthy individuals in the absence of antibiotic treatment. It is therefore clear that there are additional factors controlling the spread of AMR. Here we present data illustrating the ability of *E. coli* ST131 to outcompete and displace commensal bacteria in a germ-free mouse model. All bacterial strains used were able to colonise germ-free mice and were stable for up to 4 weeks. When co-gavaged in a 1:1 ratio the MDR ST73 and ST131 strains were able to outcompete a commensal ST10. One day subsequent to gavage the MDR strains were dominant accounting for greater than 50% of the bacterial load. The ST131 strain achieved near 100% dominance by day 4 compared to ST73 at day 6. Furthermore both ST73 and ST131 are capable of displacing a resident ST10 commensal strain. Displacement occurs both when challenging bacteria are gavaged and when mice are co-housed. ST131 produced a bacterial 'bloom' in the first 5 days of challenge. MDR strains are capable of out-competing commensals when co-gavaged into mice. Moreover MDR strains are able to dominate over resident commensals. In both instances there was no antibiotic mediated selection.

## P033

# *Galleria mellonella* as a novel *in vivo* drug discovery platform using bioluminescent *Acinetobacter baumannii*

Vanessa Francis[1], Ashley Smith[1], Christian Kemmer[2], Vincent Trebosc[2], Birgit Schellhorn[2], Richard. W. Titball[1], Olivia. L. Champion[1]
[1]BioSystems Technology, Exeter, United Kingdom. [2]BioVersys, Basel, Switzerland

## Abstract

**Background:** A major rate-limiting factor in drug development is the screening of panels of candidate drugs in a cost effective manner, before testing promising candidates in mammals. This project investigated the feasibility of using research grade *Galleria mellonella* (TruLarv) as an *in vivo* model for drug toxicity screening, for imaging and tracking pathogen growth and for assessing drug efficacy. This model has the potential to transform drug discovery by enabling high throughput drug screening *in vivo,* at an early stage in a cost effective manner.

**Materials/methods:** 10 MDR/XDR clinical isolates of *Acinetobacter baumannii* were tested in TruLarv for virulence and one isolate was engineered to constitutively express the *lux* operon. We established a protocol for visualising bioluminescent *A. baumannii* in TruLarv and for monitoring the progression of infection. Antimicrobials were tested for toxicity in TruLarv by injecting precise doses into the larvae and monitoring survival.

**Results:** We devised a protocol for screening the toxicity of antimicrobial compounds in TruLarv. We also showed that *A. baumannii* isolates had different virulence in TruLarv. One isolate was bioluminescently labelled with which we established a protocol for visualising bioluminescence from the bacteria within TruLarv and monitoring infection.

**Conclusions:** TruLarv can be used for toxicity testing of antimicrobial compounds and by using bioluminescent *A. baumannii* we were able to monitor antimicrobial efficacy in TruLarv in real time.

## P034

# Assessing antibiotic resistance and survival under aerobic stress within a panel of freshly isolated strains of *Campylobacter*.

Gillian Carney, Marguerite Clyne, Tadhg Ó Cróinín
University College Dublin, Dublin, Ireland

## Abstract

### Background

*Campylobacter jejuni* is the leading cause of bacterial diarrhoea worldwide. Contaminated chicken meat is the most common route to infection, but how this microaerophilic pathogen survives within the supermarket environment remains unclear. Another concern associated with *Campylobacter* is the rising level of fluoroquinolone resistance observed. This project aims to produce a bank of phenotypic data and identify trends aiding in the survival of *Campylobacter* and rise of antibiotic resistance.

### Methods

A panel of 153 fresh isolates from abattoir chicken, supermarket chicken and human clinical samples was established. The isolates were then assessed for motility, antibiotic resistance and tolerance to aerobic stress.

### Results

Supermarket isolates displayed significantly higher levels of motility compared to abattoir chicken and human isolates. 85% and 50% of all isolates were found to be resistant to tetracycline and ciprofloxacin respectively. Tetracycline resistance was evenly distributed across isolate groups. However, ciprofloxacin resistance was more prevalent among supermarket isolates as 80% of this isolate group were ciprofloxacin resistant. An association between oxygen tolerance and ciprofloxacin resistance was also observed, as all isolates which showed medium or high level of resistance to ciprofloxacin were also oxygen tolerant.

### Conclusion

These results highlight the prevalence of antibiotic resistance in *Campylobacter* from a range of isolate sources. The high level of ciprofloxacin resistance within the supermarket isolate group and the correlation between this resistance and oxygen tolerance also supports previous data that the supermarket environment may be selecting for fluoroquinolone resistant strains within the food chain.

## P035

# ESBL plasmid transfer by host- and country- associated E. coli within an in vitro model of the chicken caeca

Joy Leng[1], Jenny Ritchie[1], Amanda Fivian-Hughes[1], Boas Van der Putten[2], Vinh Trung Nguyen[3], Rik Oldenkamp[2,4], Martin Bootsma[4], Sumeet Kumar Tiwari[5], Sebastien Matamoros[2], Ngo Thi Hoa[3], Christian Berens[6], Julio Alvarez[7], Marta Ferrandis-Vila[6], Angelika Fruth[8], Stefan Schwarz[8], Astrid Bethe[8], Christian Menge[6], Constance Schultsz[2], Torsten Semmler[5], Roberto La Ragione[1]
[1]The University of Surrey, Guildford, United Kingdom. [2]Amsterdam UMC, Amsterdam, Netherlands. [3]The University of Oxford, Ho Chi Minh City, Vietnam. [4]UMC Utrecht, Utrecht, Netherlands. [5]Robert Koch-Institute, Berlin, Germany. [6]Friedrich-Loeffler-Institut, Jena, Germany. [7]VISAVET UCM, Madrid, Spain. [8]Freie Universitat Berlin, Berlin, Germany

## Abstract

ESBL plasmids confer antimicrobial resistance in *E. coli* and other bacteria. However, the impact of inoculating an *in vitro* model simultaneously with multiple *E. coli* isolates harbouring different ESBL plasmids has not yet been explored. Here, we inoculated a continuous flow *in vitro* model of the chicken caeca with a cocktail of 17 ESBL harbouring *E. coli* isolates. The isolates were associated with four different hosts (pig, cattle, humans and chickens) and isolated from four different countries (UK, Vietnam, Germany and Spain). The isolates were able to persist in the model during the 72 hour experiment, although the total CFU/ml for the isolates and number of individual isolates decreased over time. The presence of individual isolates within the model was assessed using ORFan gene multiplex PCR assays, targeting genes unique to each isolate. These showed that different isolates were present in the vessels for varying lengths of time, irrespective of their host-association. No trans-conjugants were detected when $10^8$ CFU/ml of the *E. coli* isolate cocktail was added to the model. However, when the vessels were inoculated with a cocktail containing $10^{10}$ CFU/ml, potential trans-conjugants were isolated in samples between taken 48 and 72 hours post addition of the *E. coli* cocktail. Here, we have shown that multiple ESBL-producing *E. coli* isolates can persist within an *in vitro* model of the chicken caeca and some of these appear able to transfer their ESBL plasmid to the pre-existing commensal *E. coli*.

**P036**

# Genomic and Phenotypic Characterisation of Multi-Drug Resistant *Escherichia coli* carried by meat handlers and slaughterhouse meat from North-Western Tanzania.

Alison MacFadyen[1], Jeremiah Seni[2], Nyambura Moremi[2], Vitus Silago[2], Stephen E. Mshana[2], Katarina Oravcová[1]
[1]University of Glasgow, Glasgow, United Kingdom. [2]Catholic University of Health and Allied Sciences, Mwanza, Tanzania, United Republic of

## Abstract

Our research is part of a larger project Supporting the National Action Plan on Antimicrobial Resistance (SNAP-AMR) in Tanzania. The aspect of this work is to determine the AMR carriage and associated mobile genetic elements of extended spectrum β-lactamase (ESBL) producing *Escherichia coli* within the community of Mwanza, in North-Western Tanzania. From 54 slaughterhouses a total of 322 *E. coli* isolates were obtained from raw meat, with 210 originating from meat handlers. Initial antimicrobial susceptibility testing identified 71 (22%) and 27 (13%) of the meat and meat handler isolates, respectively, as being ESBL *E. coli*. ST2852 was the most abundant MLST, followed by globally disseminated and ESBL associated ST38. Whole genome sequencing and phenotypic analysis revealed 88% of our isolates were resistant to three or more antimicrobial classes. On average, our isolates carried at least 10 different AMR associated genes, with 80% having one or more AMR conferring chromosomal mutation. In relation to ESBL, 88 out of 98 of the isolates carried the globally disseminated allotype $bla_{\text{CTX-M-15}}$, with over 55% of the isolates encoding $bla_{\text{CTX-M-15}}$ on multi-drug resistant (MDR) plasmids. The remaining strains encoded one of three different $bla_{\text{CTX-M}}$ allotypes. Our data highlights high prevalence of MDR ESBL *E. coli* within the community and its potential risk of transmission through the food chain. In our future work, we will combine these community carriage data with our work on ESBL Enterobacteriaceae carriage and infection within healthcare settings, to evaluate the genomic epidemiology of ESBL organisms in this region.

## P037

# Undetected transmission of Gram negative organisms occurring within a neonatal unit despite routine screening

Vivien Price[1], Alan McNally[1], Jonathan Swindells[2], Steven Dunn[1]
[1]University of Birmingham, Birmingham, United Kingdom. [2]The Royal Wolverhampton NHS Trust, Wolverhampton, United Kingdom

## Abstract

Background

Gram negative infections cause significant morbidity and mortality in neonates, with antimicrobial-resistant (AMR) pathogens of particular concern. Many neonatal units routinely screen for colonisation. We aimed to characterise the presence of any transmission events not being captured by routine screening.

Methods

Faecal and umbilical samples were collected weekly for 2 months in a 32-bed unit. 155 isolates of *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella oxytoca* and *Klebsiella pneumoniae* from 44 neonates were sequenced using the Illumina platform.

Results

15/44 neonates were involved in 8 putative transmission clusters, with clusters involving 1-4 neonates seen for all 4 studied species. For 6/8 putative clusters, 1-8 SNPs separate isolates from different neonates. All clusters included one or more AMR genes, including betalactamases, aminoglycoside phosphotransferases and genes encoding for tetracycline, oxyquinolinine and Fosfomycin resistance.

No neonate had a previous or subsequent Gram negative bacteraemia. Overall length of stay in days (LOS) (range 2-84, median 11) was longer in those involved in 1 cluster (median 32) or 2 clusters (median 78). Pre-term birth, invasive ventilation, necrotizing enterocolitis diagnosis and parenteral nutrition also appeared to be associated with involvement in a transmission cluster.

Conclusions

Transmission of organisms was widespread, occurred across different species, and included AMR genes of potential clinical relevance, though no consequent clinical infections were observed. None of these clusters had been detected by the hospital's routine Gram negative screening. The results suggest repeated breakdowns in infection control resulting in person-to-person transmission in this setting.

## P038

# The Missing Link: Developing a pipeline for accelerated antibiotic discovery from *Streptomyces* through linking 'omics data.

Darren Scobie[1], Paul Herron[1], Andrew Ramsay[2], Grimur Hjorleifsson Eldjarn[2], Simon Rogers[2], Katherine R Duncan[1]
[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom

## Abstract

The bacterial genus *Streptomyces* is unsurpassed in their ability to produce bioactive specialised metabolites, accounting for approximately 80% of all microbially-produced antibiotics. Recent strategies to combine comparative genomics and metabolomics data have been shown to accelerate novel metabolite discovery, while expanding our biosynthetic and chemical understanding of these microorganisms.

In this study, ten *Streptomyces* strains, including *S. fradiae, S. spectabilis* and *S. vinaceus* were selected based on phylogeny and genome sequence data. Genome mining revealed a total of 326 Biosynthetic Gene Clusters (BGCs), spanning 21-45 per strain. Interestingly, despite being well studied, 12% of these BGCs showed less than ten percent sequence similarity to known BGCs, indicating potential novelty. The culture of these strains has been optimized, utilizing a range of minimal media to maximise chemical diversity. Preliminary results indicate that metabolite culture extracts of *S. spectabilis* and *S. nodosus* showed antibiotic activity against *B. subtilis* and *E. coli* at a concentration of 1 mg/mL and have therefore been prioritised for LC-MS/MS analysis. The metabolite profiles generated will be linked to genome mining data using a standardised scoring method based on strain presence and absence to prioritise novel specialised metabolites.

## P039

# IMP-1700 promotes the bactericidal activity of daptomycin

Amber Sefton[1], Elizabeth Ledger[1], Thomas Lanyon-Hogg[2], Ed Tate[1], Andrew Edwards[1]
[1]Imperial College London, London, United Kingdom. [2]University of Oxford, Oxford, United Kingdom

## Abstract

IMP-1700 is a small-molecule that has recently been shown to reverse quinolone resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) by inhibiting the AddAB bacterial DNA repair complex. However, IMP-1700 also exhibited antibacterial activity, despite the non-essential nature of AddAB, indicating an additional target for the compound. It was found that IMP-1700 disrupted the staphylococcal membrane, causing permeabilisation and increased fluidity. IMP-1700 also increased the binding of the antibiotic daptomycin to the staphylococcal membrane, resulting in increased bacterial killing. This effect appeared to be due to the inhibition of the release of membrane phospholipids, which we have previously shown sequester the antibiotic and enable bacterial survival. In summary, IMP-1700 is a novel antibiotic that exhibits membrane-disrupting activity against MRSA and enhances daptomycin activity. Work is ongoing to identify the target of IMP-1700.

## P040

# Evidence for universal dynamics of antimicrobial resistance genes in urban sewage: a global study

Leonie Maier, Luke McNally, Mark Woolhouse
University of Edinburgh, Edinburgh, United Kingdom

## Abstract

The extent to which the global spread of antimicrobial resistance genes (AMR) is limited by dispersal processes is critical for tackling this global public health threat and limit the spread of emerging AMR genes. We approached this question by assessing the "universality" of global AMR gene dynamics. Universal dynamics implies that the same sets of AMR genes are selected for in all locations, with inter-location variability driven by differences in colonising AMR genes.

Using data from the Global Sewage Surveillance Project (GSSP) we assessed if there was a universal dynamic signature in the global AMR in human associated microbiomes. The GSSP samples AMR genes from 79 sites in 60 countries. We evaluated the relationship between the overlap (the species two samples have in common) and dissimilarity (the root Jensen-Shannon divergence distance metric between these two samples) of AMR genes using multiple-membership mixed effects models. We found consistent evidence for a negative relationship between dissimilarity and overlap of AMR gene compositions, implying universal dynamics. This relationship held while controlling for geographic separation, socio-economic factors, and antimicrobial usage practices. Together these results suggest that dispersal is a critical factor determining the global distribution of AMR genes.

Our results show that the old adage "everything is everywhere but the environment selects" does not hold true for AMR genes, implying that dispersal barriers play a critical role in shaping the worldwide AMR gene pool. This suggests that efforts to limit dispersal of newly emerging AMR genes could critically limit their global spread.

## P041

# Possible role of L-form switching in recurrent urinary tract infection

Katarzyna Mickiewicz[1], Yoshi Kawai[2], Lauren Drage[2], Margarida C Gomes[3], Frances Davison[2], Robert Pickard[2], Judith Hall[2], Serge Mostowy[3], Phillip D Aldridge[2], Jeff Errington[2]
[1]Newcastle University, Newcatle upon Tyne, United Kingdom. [2]Newcastle University, Newcastle upon Tyne, United Kingdom. [3]London School of Hygiene and Tropical Medicine, London, United Kingdom

## Abstract

Recurrent urinary tract infection (rUTI) is a major medical problem, especially in the elderly and infirm, but the nature of the reservoir of organisms responsible for survival and recolonisation after antibiotic treatment in humans is unclear. Here, we demonstrate the presence of cell-wall deficient (L-form) bacteria in fresh urine from 29 out of 30 older patients with rUTI. In urine, E. coli strains from patient samples readily transition from the walled state to L-form during challenge with a cell wall targeting antibiotic. Following antibiotic withdrawal, they then efficiently transition back to the walled state. E. coli switches between walled and L-form states in a zebrafish larva infection model. The results suggest that L-form switching is a physiologically relevant phenomenon that may contribute to the recurrence of infection in older patients with rUTI, and potentially other infections.

## P042

# How does growth environment affect antimicrobial susceptibility to antibiotics?

Robynn Wiszniewska, Freya Harrison, Jenny Littler
School of Life Sciences, University of Warwick, Coventry, United Kingdom

## Abstract

Antimicrobial resistance (AMR) is a leading cause of death worldwide and a major public health threat. *Pseudomonas aeruginosa* is a problematic pathogen with high incidences of AMR, particularly in biofilms present in chronic infections of nonhealing wounds and the lungs of people with cystic fibrosis (CF). Antimicrobial susceptibility testing (AST) performed in media that does not reflect the host environment often produces inaccurate results that fail to correlate with patient outcome, suggesting environment affects antimicrobial susceptibility.

*P. aeruginosa* strains PA14 and LESB58 were cultured with serial dilutions of meropenem in different media – standard Mueller-Hinton broth (MHB), synthetic cystic fibrosis sputum media (SCFM) and synthetic wound fluid (SWF). Minimum inhibitory concentrations (MICs) were lower when assessed in SCFM and SWF compared with MHB. This was particularly evident in the CF isolate LESB58, which was resistant in MHB but susceptible in SCFM and SWF, according to EUCAST guidelines. In an *ex vivo* pig lung model of CF biofilm, the meropenem MIC determined in SCFM reduced the growth of *P. aeruginosa* by one $\log_{10}$ but failed to inhibit growth entirely.

In conclusion, the media used in AST has a substantial effect on bacteria and the resulting MIC. This highlights the need to further investigate the role of environment on antibiotic susceptibility, in order to develop more appropriate and accurate diagnostic tests which target specific antibiotics to ensure effective bacterial clearance. This is important in limiting bacterial exposure and allows the effective stewardship of current antibiotics as part of the battle against AMR.

## P044

# Impact of water hardness on microbial diversity and carbapenemase-producing members of the Enterobacterales of hospital sink drain traps

Nadia Mohammed[1], Jim Huggett[1,2], Ginny Moore[3], Jennifer Ritchie[1]
[1]University of Surrey, Guildford, United Kingdom. [2]National Measurement Laboratory, Teddington, United Kingdom. [3]Biosafety, Air and Water Microbiology Group, Public Health England, Porton Down, United Kingdom

## Abstract

Multiple reports have linked hospital hand-washing sink waste traps as a possible reservoir for antibiotic resistant bacteria including carbapenemase-producing Enterobacterales (CPE). CPE acquisition primarily occurs among hospitalised patients (mortality rate 56.7%). Although multiple risk factors have been identified in CPE transmission, several outbreaks have been linked to hospital sinks. The aims of our study were to investigate bacterial communities in hospital sink waste traps, to explore their diversity and CPE content under soft and hard water conditions and the potential for resistance gene transmission of extracted CPE isolates *in vitro*. Waste traps, colonised with CPE, were obtained from a UK hospital, and fitted into a model sink system with an automated water flushing regimen. Biofilm and wastewater samples were collected at the time of installation and weekly thereafter for 12 weeks. After 6 weeks, water hardness from automated faucets increased from 10mg to ~220mg $CaCO_3$/L. Samples were subject to culture-based quantification and genomic extraction for metagenetic and whole genome comparisons. Culture-based analyses showed heterotrophs and CPE concentrations were not significantly increased or decreased by changes in water chemistry; metagenetic analysis demonstrated complex, diverse microbiomes. Whole genome sequencing of KPC-positive *Enterobacter cloacae* identified strains belonging to sequence types (ST) 252, 87 and 32. *In vitro* assays proved isolates produced greater biofilm under low versus high calcium water conditions, but similar differences were not observed in nutrient-rich conditions. These findings suggest water chemistry may be implicated in biofilm formation by CPE, which could sequentially impact the spread of resistance in sink communities.

## P045

# Biocide adaptation in urinary tract pathogens: the clinical relevance of reduced chlorhexidine minimum inhibitory concentration in *Proteus mirabilis*

Ocean E Clarke[1], Harriet Pelling[1], Jonathan N Nzakizwanayo[1], Takanori Matsumoto[2], Anthony J Slate[1], Susanne Gebhard[1], Matthew E Wand[3], J Mark Sutton[3], Brian V Jones[1]
[1]University of Bath, Bath, United Kingdom. [2]Tokyo University of Agriculture and Technology, Tokyo, Japan. [3]Public Health England, Salisbury, United Kingdom

## Abstract

*Proteus mirabilis* is a leading cause of catheter-associated urinary tract infection (CAUTI), forming extensive crystalline biofilms on urethral catheters that can occlude urine flow, leading to serious medical complications and a significant financial burden on healthcare services worldwide. Products containing the cationic membrane active biocide chlorhexidine (CHD) are often used in attempts to control CAUTI. However, some *P. mirabilis* clinical isolates exhibit CHD MICs above the in-use concentration found in catheter management products *e.g.* Uro-Tainer® (200 μg $mL^{-1}$ CHD), a bladder irrigation solution available on the NHS supply chain. To determine if CHD MICs were predictive of clinically relevant resistance to the Uro-Tainer® irrigation solution, *in vitro* bladder model experiments were conducted using clinical isolates of *P. mirabilis:* RS50a (CHD MIC: 8 – 16 μg $mL^{-1}$) – an isolate which is highly susceptible to CHD, RS47 (CHD MIC: > 512 μg $mL^{-1}$) – a CHD 'resistant' isolate, and RS28 (CHD MIC: 128 – 256 μg $mL^{-1}$) – an isolate with intermediate CHD susceptibility. Despite differences in MIC, CHD Uro-Tainer® treatment significantly increased blockage time in all three isolates and blockage time following treatment was similar across the three isolates. Interestingly, no viable planktonic cells were recovered from any of the three isolates immediately following Uro-Tainer® treatment, suggesting that CHD treatment may be forcing cells into a viable but non-culturable state. Alternatively, surviving biofilm-associated cells could be reseeding the planktonic cell population. The data throughout this study demonstrated that the CHD MIC for *P. mirabilis* does not provide a reliable indicator of clinical susceptibility.

## P046

# Characterising MmyJ, an ArsR-like transcriptional regulator that controls antibiotic resistance

Rohini Ajaykumar, Christophe Corre, Allister Crow
The University of Warwick, Coventry, United Kingdom

## Abstract

The model organism and antibiotic maker *Streptomyces coelicolor* A3(2) contains a natural plasmid, SCP1, where all the genes for production and resistance of the antibiotic methylenomycin are encoded [1–3]. While certain proteins from this cluster have been characterised in varying detail, the transcriptional regulator that controls methylenomycin resistance, MmyJ,) remains poorly understood [4]. Through overproduction in *Escherichia coli* and purification to high concentrations, the function of recombinant MmyJ are currently being investigated. Electromobility shift assays using a fragment of DNA between the *mmr* and *mmyJ* genes show binding of MmyJ. Crystal screens for X-ray crystallography have yielded crystals of MmyJ that will be used to unravel structural details allowing for insights into the structure-function of MmyJ. Unlike other ArsR-like regulators, MmyJ is believed to sense an organic molecules as opposed to metal ions. Analysis of transcriptional regulators like MmyJ will contribute to knowledge on antibiotic resistance mechanisms and aid the development of an inducible system.

References:

1. WRIGHT, L. F. & HOPWOOD, D. A. Actinorhodin is a Chromosomally-determined Antibiotic in Streptomyces coelicolor A3(2). *J. Gen. Microbiol.* **96**, 289–297 (2009).
2.Lane, C. & Nr, N. Genetic Determination of Methylenomycin Synthesis by the. **3**, 239–252 (1977).
3. Chater, K. F. & Bruton, C. J. Resistance, regulatory and production genes for the antibiotic methylenomycin are clustered. *EMBO J.* **4**, 1893–1897 (1985).
4. Lougher, M. Functional and structural insights into MmyJ, An ArsR-Like Transcriptional Repressor. (2015).

## P047

# A simple, iterative method for screening snake venoms to identify proteins that have antibacterial activity towards *Escherichia coli*

Alice J Fraser[1,2], Mark Wilkinson[1], Nicholas Casewell[1], Adam P Roberts[1], Alasdair T M Hubbard[1]
[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]London School of Hygiene and Tropical Medicine, London, United Kingdom

### Abstract

Due to the incidence of antimicrobial resistant bacteria increasing, and lack of antibiotic development, novel antibiotics are desperately needed. The diversity of proteins found within snake venom make them an attractive resource for drug discovery, having already demonstrated therapeutic potential as treatment for hypertension. Snake venom proteins have also previously shown to have antibacterial activity and therefore represent an underexplored resource for antibiotic discovery.

Iterative testing of whole, dialysed (into PBS) and fractionated (by cation exchange liquid chromatography) snake venom, as well as purified proteins from *N. mossambica*, was performed in several stages using an in-house developed assay to detect antibacterial activity towards *Escherichia coli*.

Initial testing of 14 whole snake venoms identified eight with significant antibacterial activity towards *E. coli*. Following dialysis, six of the eight venoms' antibacterial activity was confirmed to be due to proteins, rather than peptides. Venom from the well characterised *N. mossambica* and less well characterised *N. melanoleuca* were fractionated and assayed, of which 29 and 19 protein fractions had significant activity, respectively. Antibacterial activity of two major protein classes from *N. mossambica* was assessed, confirming the previously known antibacterial activity of the three-fingered cytotoxin 3 and basic phospholipase A2, corresponding to the activity seen in fractions 24-26 and 30-31, respectively.

We have devised a simple, iterative method that is able to identify proteins within snake venom that have known antibacterial activity. We are also able to identify protein fractions with antibacterial activity caused by unidentified proteins, which will be explored further.

## P048

# Identification of Novel Combinatorial Drug Targets in Mycobacterium tuberculosis

Pooja -[1], Alex Smith[2], Thomas Mendum[1], Rachel Butler[1], Suzie Hingley-Wilson[1], Graham Stewart[1]
[1]University of Surrey, Guildford, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom

## Abstract

Tuberculosis caused by the *Mycobacterium tuberculosis* complex (MTBC) remains one of the most important infectious diseases of mankind. Isoniazid (INH) and rifampicin are the main first line drugs used in multidrug treatment of TB but phenotypic tolerance and the development of resistance against these compounds and other TB drugs is a serious and increasing problem. The overall aim of this project is to utilise transposon sequencing (Tnseq) genetic screens of MTBC to identify novel drug targets that function maximally when in combination with INH or rifampicin or increase the sensitivity to these drugs. Drugs directed to these targets could be developed as part of new multidrug therapies. INH is a prodrug which requires activation by the mycobacterial catalase/peroxidase, KatG to form an isonicotinoyl acyl radical, which binds to $NAD^+$/NADH and inhibits enoyl-[acyl-carrier-protein] reductase (InhA)-dependent synthesis of cell wall mycolic acid. A Tnseq screen of BCG in a sub-MIC concentration of INH identified genes whose disruption increased apparent sensitivity to INH. A subset of these genes were functionally related to oxidative stress and were selected for further investigation of individual wild-type strains, mutants and complemented mutants. KatG activity was measured with a catalase assay to test the hypothesis that absence of the oxidative stress gene leads in the production of more KatG which allows for increased activation of the drug INH and increased sensitivity to INH. In a second Tnseq screen, mutants were identified with enhanced sensitivity to rifampicin.The significance of these genes will be investigated further and discussed.

## P049

# Conserved distribution of Biosynthetic Gene Clusters in the genus *Micromonospora*

David Mark[1], Cristina Dorador[2], Nicholas Tucker[1], Paul Herron[1]
[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Antofogasta, Antofogasta, Chile

## Abstract

Antimicrobial resistance (AMR) is an ever-increasing threat, with some estimates predicting that the year 2050 will see 10 million people dying annually to an AMR infection. One method to combat this is the discovery of new antimicrobial natural products, enabled in part by "genome-mining", where an organism of interest's genome is scanned for the presence of biosynthetic gene clusters (BGCs). As whole-genome-sequence data for an ever increasing number of species is made available, so too is the opportunity for comparing the BGC content of these species . Here, using circular, single-contig genomes from the genus *Micromonospora*, we sought to test if there is a conserved distribution of BGCs on the chromosome. To achieve this, we reoriented 25 *Micromonospora* chromosome assemblies to begin at the origin of replication (*oriC*), and mapped the location of the BGCs present in each to a linear pseudochromosome. This mapping revealed a "W" shaped distribution of BGCs on the chromosome, with BGCs clustering at either side of the origin of replication as well as at the terminus of replication, *ter*. Additionally, there was a difference in the BGC content of the three peaks: there was less diversity in *oriC*-proximal BGCs than in *ter*-proximal BGCS. We illustrate that the distribution of BGCs on the circular chromosome of *Micromonospora* is nonrandom, and thus highlight the importance of mining high-quality assemblies of bacterial genomes to gain insight into the genomic architecture of secondary metabolism.

## P050

# Molecular study and prevalence of *oqxAB*-bearing plasmids in *Escherichia coli*, a widespread multidrug-resistant bacterium in human, animal, and the environment

Andes LAU, Huiluo CAO, Man-Ki TONG, Kin-Hung CHOW, Pak-Leung HO
The University of Hong Kong, Hong Kong, Hong Kong

## Abstract

**Background:**

Antibiotic 'olaquindox' was used as feed additive in food animal production, resulting in the wide spread of *Escherichia coli* with plasmid encoding multidrug-resistant efflux pump OqxAB in animal, human and environment. Considering its possible impact on public health, thorough understanding and constant monitoring are necessary.

**Objective:**

This project aims to investigate the prevalence, virulence, and phylogenetics of *E. coli* carrying *oqxAB*-plasmid.

**Method:**

Next Generation Sequencing (NGS) analysis of *E. coli* with IncFII plasmid encoding *oqxAB* isolated from human (n=21) and livestock (n=27) was performed. Further *in vivo* virulence study conducted using *E. coli* J53 transconjugants carrying *oqxAB*-bearing plasmid and *Galleria mellonella* larvae as infection model. Meanwhile to study the prevalence of *E. coli* carrying *oqxAB*, animal (n=184), meat (n=729), and environmental (n=103) samples were screened via phenotypic and molecular characterization between March 2019 and February 2021.

**Result:**

NGS investigation indicated F18 as the major allele among the studied plasmids (n=27), while F16 (n=12), F33 (n=6), F24 (n=2), and F14 (n=1) were also found. Similar set of virulence factors were observed in plasmids of same Inc allele, and multiple antibiotic resistance genes co-carriage was common. *in vivo* testing showed significant health deterioration, but fatality, caused by the transconjugants. Among the screened samples, *E. coli* carrying *oqxAB*-plasmid was isolated from 54 animal, 99 meat, and 7 environmental samples.

**Conclusion:**

In conclusion, *oqxAB*-plasmids identified from animal and human are highly correlated, and the wide spread of it is a public health concern due to the increased pathogenicity and multidrug-resistant nature.

## P051

# Bioengineered derivatives of nisin display enhanced antimicrobial activity against S. epidermidis

Ellen Twomey[1], Colin Hill[2], Des Field[2], Máire Begley[1]
[1]Munster Technological University, Cork, Ireland. [2]University College Cork, Cork, Ireland

## Abstract

Its ubiquitous nature and ability to form biofilms on biotic and abiotic surfaces has marked *S. epidermidis* as a serious nosocomial threat. We investigated the ability of the lantibiotic nisin A for antimicrobial activity against 18 strains of *S. epidermidis* obtained from a hospital setting. A bank of 29 rationally selected bioengineered nisin derivative-producing organisms were also investigated to identify derivative peptides with enhanced bioactivity. Agar-based deferred antagonism assays confirmed that nisin A could inhibit the growth of all *S. epidermidis* strains tested. Several derivative producing strains, (VGA, VGT, SGK, M21A, M17Q, AAA), caused zones which were significantly larger than those produced by the wildtype against all 18 *S. epidermidis* strains, suggesting enhanced activity. A broth-based MIC assay performed with purified peptides verified the enhanced activity of M17Q, as it displayed a twofold lower MIC than nisin A against two separate *S. epidermidis* strains. Biofilm-inhibition assays performed on untreated plastic microtiter plates and medical device materials, (stainless steel and the catheter materials, PVC, PVR and rubber), indicate that both the wildtype peptide and M17Q peptide could significantly reduce biofilm formation on all surfaces investigated. However, M17Q showed enhanced biofilm inhibition capabilities on plastic and stainless-steel compared to nisin A. Incubation of *S. epidermidis* with either nisin A or M17Q in simulated wound fluid demonstrated that both peptides retained their activity, with M17Q also maintaining its enhanced antimicrobial effects. The findings of this study show the potential of lantibiotics and their bioengineered derivatives in the control of medical device-associated *S. epidermidis* infections.

## P052

# Investigating *Streptomyces clavuligerus* Linear Replicons for Improved Clavulanic Acid Production

Elmira Mohit[1], Paul Herron[1], Ben Huckle[2], Steve Kendrew[2]
[1]University of Strathclyde, Glasgow, United Kingdom. [2]GlaxoSmithKline, Worthing, United Kingdom

## Abstract

Antibiotic resistance in pathogenic microbes has been a recognised problem for the last 50 years, including resistance from ß-lactamase producing bacteria against ß-lactam antibiotics. ß-lactamase producing bacteria have prompted research into inhibitors of this enzyme; one of which is clavulanic acid, used in combination with amoxicillin, to form the drug Augmentin®. Clavulanic acid is produced by *Streptomyces clavuligerus* (*Sclav*), which has a dynamic genome architecture composed of four giant linear plasmids (GLPs) in addition to its chromosome. This project aims to investigate plasmid-chromosome interactions to ultimately cure GLPs and increase clavulanic acid production by decreasing the metabolic burden generated by these GLPs.

Various genes are essential for the maintenance of linear replicons, such as *tap* and *tpg* which encode telomeric terminal proteins. Previous work demonstrated that the absence of Tap and Tpg from one replicon caused chromosomal shortening, leading to replicon circularisation. We will further determine the significance of these genes through their inactivation by the employment of CRISPR-dcas9 and CRISPR-BEST. These systems 'silence' genes, either stopping transcription or introducing a stop codon; they remove the necessity for repair of replicon double stranded breaks required by other targeted mutagenesis systems in this poorly recombinogenic strain.

Through characterisation of the interactions of Tap and Tpg with palindromic sequences at the replicon ends of *Sclav* we will elucidate the mechanism of telomere replication in this complex multi-replicon organism. This will allow us to better understand the genome interactions of *Sclav* enabling the production of genetically engineered strains with increased clavulanic acid productivity.

## P053

# Evolutionary trade-offs impact antimicrobial resistance within Pseudomonas aeruginosa populations sourced from cystic fibrosis lungs

Jelly Vanderwoude[1], Sheyda Azimi[1], Timothy Read[2], Stephen P. Diggle[1]
[1]Center for Microbial Dynamics and Infection, School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA. [2]Division of Infectious Diseases, Department of Medicine, Emory University, Atlanta, GA, USA

## Abstract

*Pseudomonas aeruginosa* (*Pa*), the predominant pathogen in chronic lung infection of adults with cystic fibrosis (CF), possesses a number of mechanisms which contribute to antimicrobial resistance (AMR). Even aggressive antibiotic treatment is ineffective at clearing chronic *Pa*, which may partially be due to the rapid diversification displayed by *Pa* during long-term CF infection. However, our knowledge of the role of population heterogeneity on AMR is limited, as prior studies have chronically under-sampled *Pa* isolates in CF lungs. Specifically, the role of evolutionary 'trade-offs' on AMR in these patients has been overlooked. We sampled 75 *Pa* isolates from expectorated sputum samples of four CF adults chronically infected with *Pa* (n = 300) and tested each for growth rate in lysogeny broth and synthetic CF sputum media (SCFM1), susceptibility profiles to six antibiotics commonly prescribed to CF patients, and surface attachment to assess the role of population heterogeneity on AMR. We found significant within-patient heterogeneity in AMR across all patients and antibiotics. The majority of isolates were well within the range of susceptibility for the tested antibiotics, despite ineffective clearing of *Pa* infection for each of these patients. When looking across all four patients, we identified potential evolutionary trade-offs between antimicrobial resistance and growth rate for the following antibiotics: amikacin, meropenem, ciprofloxacin, and tobramycin. Conversely, we found no significant relationship between growth rate and surface attachment, nor AMR and surface attachment. Overall, these results suggest that growth rate may trade-off with AMR to sustain diverse populations resistant to disturbances in CF lungs.

## P054

# Characterising resistance breaking molecules for the treatment of *Pseudomonas aeruginosa* infection

Frèdi Langendonk
University of Liverpool, Liverpool, United Kingdom

## Abstract

*P. aeruginosa* is a nosocomial pathogen capable of causing persistent, multi-drug resistant infections due to its many intrinsic, acquired and adaptive resistance mechanisms against clinically used antibiotics. The WHO categorized *P. aeruginosa* as a priority one pathogen, meaning it is critical new drugs or alternative therapies are developed against *P. aeruginosa* infection. Previous work has identified a missense mutation associated with increased antibiotic susceptibility in the *pmrB* histidine kinase gene of the PmrAB two-component signaling system (TCSS). Combination therapy with antibiotic potentiating adjuvants targeting TCSS like PmrAB have the potential to overcome resistance by enhancing the antibiotic agent against highly resistant *P. aeruginosa*.

The aim of this research is to develop such adjuvant therapy to optimize the use of antibiotics in the treatment of *P. aeruginosa* infection. A high-throughput screening method has been designed to identify antibiotic adjuvants with a broad spectrum of activity by screening a compound library with sub-MIC concentrations of two different classes of antibiotics, colistin (polymyxin) and tobramycin (aminoglycoside). In total, 19/2480 compounds screened were found to act as potentiators for both antibiotics. Initial screening has identified both antibiotic potentiating adjuvants (10/19) and directly antimicrobial molecules (9/19) with low levels of cytotoxicity in A549 lung epithelial cells. Four of these lead compounds are characterized further, by testing activity against a range of *P. aeruginosa* isolates, in biofilm models, and in the presence of serum. In addition, potentiator targets within the bacterial cell will be identified with the use of Drug affinity responsive target stability (DARTS), and molecular docking simulations.

## P055

# Profiling the effects of acne therapeutics, including the novel antibiotic sarecycline, on the human microbiota

Anthony Buckley, Ines Moura, Emma Clark, James Altringham, William Spittal, Karen Bentley, Mark Wilcox
University of Leeds, Leeds, United Kingdom

### Abstract

Many factors shape the human intestinal microbiota, some of which can confer a deleterious effect on the microbiota, e.g. antibiotic therapy. Disruption to the microbiota has been implicated in the progression of *C. difficile* infection (CDI), multiplication of multi-drug resistant organisms and many extra-intestinal diseases. Thus, determining the off-target effects of antibiotics is essential to determine a patient's risk of these diseases, particularly for new therapies. Here we characterise the effect of sarecycline, a novel tetracycline antibiotic for the treatment of moderate to severe acne vulgaris; and compared its effect to the gut microbiota with other acne treatments. Using four independent *in vitro* gut models, we exposed the human microbiota to either sarecycline, minocycline, doxycycline, or clindamycin, and monitored the changes to the bacterial populations, and whether these changes were sufficient to induce CDI.

Sarecycline or doxycycline exposure caused a temporary reduction in the bacterial diversity upon initial exposure. Sarecycline exposure was characterised by a transient increase in *Enterococcus* spp. and Enterobacteriaceae, and a decrease in *Bifidobacterium* spp. Doxycycline exposure caused longer-term changes to the Lactobacillaceae and Ruminococcaceae populations. Minocycline exposure resulted in a dramatic reduction to the bacterial diversity, with extensive expansions to the *Enterococcus* spp. and Enterobacteriaceae populations, whilst the Lactobacillaceae and Ruminococcaceae populations contracted. Whilst clindamycin did induce simulated CDI, neither sarecycline, minocycline, nor doxycycline created a niche conducive for CDI.

These data show that long-term sarecycline use has a lower potential for disruption of the colonic microbiota, compared with the current treatments for acne vulgaris.

## P056

# Colonisation and development of antibiotic tolerance of *Pseudomonas aeruginosa* biofilms in an *ex vivo* model of cystic fibrosis lung infection

Freya Allen, Freya Harrison, Niamh Harrington
School of Life Sciences, University of Warwick, Coventry, United Kingdom

## Abstract

*Pseudomonas aeruginosa* is a primary pathogen of the cystic fibrosis airway and the resulting chronic biofilm infections are the leading cause of lung function decline and eventual mortality in people with cystic fibrosis. The cause of the switch from initial, acute infection to these established chronic infections remains to be fully understood and is key in prognosis. To investigate this switch, we have measured the development of antibiotic tolerance and production of virulence factors such as protease over a time course infection of *P. aeruginosa* in an *ex vivo* pig lung infection model. Pig lungs were dissected, infected with *P. aeruginosa*, and surrounded with artificial sputum media to model cystic fibrosis lungs. Both clinical isolates and lab isolates were used for comparison and tolerance to antibiotics was measured at different time points. Gene expression patterns over the infection time course in the lung model were also analysed, using RNA sequencing to identify levels of virulence factors and antibiotic resistance associated gene expression. Identifying differential expression of these key infection characteristics will provide further insight into the transition of an acute infection to a chronic infection in the cystic fibrosis lung. By understanding the infection progression, it will allow the development of better treatment options.

## P057

# a novel Staphylococcus aureus membrane protein MspA, that affects toxin secretion and β-lactam resistance.

Alaa Abdulaziz Alnahari[1], Seána Duggan Duggan[1], Maisem Laabei[2], Tarcisio Brignoli Brignoli[1], Ruth Massey[1]
[1]School of Cellular and Molecular Medicine, University of Bristol, Bristol, United Kingdom. [2]Department of Biology and Biochemistry, University of Bath., Bath, United Kingdom

## Abstract

Staphylococcus aureus is a major human pathogen. It can cause a variety of infections, ranging from superficial skin infection to severe infections such as sepsis and bacteraemia. Treatment is problematic due to the rise of antibiotic resistance, and so new methods to prevent and treat infections are needed. To develop these, we need to better understand how these bacteria cause diseases. One approach is to apply functional genomics, which has identified a novel membrane-bound protein called MspA. Inactivation of the gene encoding MspA results in a reduction in cytolytic toxin secretion and increased β-lactam resistance.

## P058

# ApmA is a Unique Aminoglycoside Modifying Enzyme Capable of Inactivating Apramycin

Emily Bordeleau[1], Peter J. Stogios[2,3], Elena Evdokimova[2,3], Kalinka Koteva[1], Alexei Savchenko[2,3,4], Gerard D. Wright[1]
[1]David Braley Centre for Antibiotics Discovery, M.G. DeGroote Institute for Infectious Disease Research, Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Canada.
[2]Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Canada.
[3]Center for Structural Genomics of Infectious Diseases (CSGID), University of Calgary, Calgary, Canada.
[4]Department of Microbiology, Immunology and Infectious Diseases, University of Calgary, Calgary, Canada

## Abstract

Apramycin is currently in development as a next-generation aminoglycoside antibiotic to combat major drug resistant pathogens prioritized by the World Health Organization. The unique structure of this antibiotic evades the known clinically relevant aminoglycoside resistance mechanisms. The goal of our work is to investigate the most recently identified genetic element found to confer apramycin resistance, *apmA*.

ApmA is an acetyl-CoA-dependent aminoglycoside acetyltransferase. Unlike most other such enzymes that belong to the canonical GCN5-superfamily, ApmA shows a high degree of similarity to the left-handed β-helix (LβH) acetyltransferases family of proteins. Other enzymes from this family are only known for their inactivation of the antibiotics chloramphenicol and Type A streptogramins through *O*-acetylation. Crystallographic analysis of ApmA confirmed that this enzyme adopts the predicted trimeric LβH fold and preserved active site architecture. Crystal structures were obtained with four different aminoglycosides to identify key molecular interactions in the active site. Residues responsible for substrate recognition and catalysis were investigated through site-directed mutagenesis. Antimicrobial susceptibility testing and *in vitro* enzyme assays verified that ApmA *N*-acetylates apramycin and other aminoglycosides that are clinically important. ApmA was also found to acetylate apramycin with a regiospecificity not seen for other apramycin modifying enzymes.

Our investigation provides the first structural evidence of an LβH acetyltransferase capable of aminoglycoside modification. With *apmA* detected in both Gram-positive and Gram-negative pathogens, this work provides invaluable knowledge for evading this potential form of clinical resistance.

## P059

# Evaluation of essential oils efficacy on antibiotic-resistant Salmonella strains

Chiara Masotti[1], Roberta Battistini[1], Valeria Listorti[2], Elisabetta Razzuoli[2], Clara Tramuta[3], Lucia Decastelli[3], Carlo Ercolini[1], Laura Serracca[1]
[1]Istituto zooprofilattico del Piemonte, Liguria e Valle d'Aosta, La spezia, Italy. [2]Istituto zooprofilattico del Piemonte, Liguria e Valle d'Aosta, Genova, Italy. [3]Istituto zooprofilattico del Piemonte, Liguria e Valle d'Aosta, Torino, Italy

## Abstract

**Background:** The emergence of multidrug resistant (MDR) pathogens has drawn attention to natural antimicrobial compounds, such as essential oils (EOs)

**Methods:** The aim of the present study was to investigate the inhibitory activity of three EOs against 15 *Salmonella* strains resistant to 16 commonly used antimicrobials. EOs of *O. vulgare, T. serpyllum,* and *T. vulgaris* were tested in triplicate for 15 bacterial strain by Kirby-Bauer disk diffusion method and broth microdilution method to determine the minimal inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), according to the Clinical and Laboratory Standards Institute guidelines with minor modifications.

**Results:** *O. vulgare* and *T. serpyllum* showed high antimicrobial activity compared to *T. vulgaris* in all tested antibiotic-resistant strains (*P*-value <0.01). The mean diameter of the bacterial growth inhibition zone was 18.7 mm for *O. vulgaris*, 19.2 mm for *T. serpyllum*, and 14.2 mm for *T. vulgaris*. The MIC and MBC mean values of *O. vulgare* were respectively 0.037% and 0.058%, for *T. serpyllum* 0.036% and 0.041%, for *T. vulgaris* 0.13% and 0.15%. All strains resulted resistant to trimethoprim-sulamethoxazole and 14 strains resulted resistant to tetracycline, considered a highly important antimicrobial. Among *Salmonella* serovars tested the monophasic *S. Typhimurium* has exhibited the highest antimicrobial resistance to synthetic molecules while they were sensitive to EOs.

**Conclusions**: The EOs of *O. vulgare* and *T. serpyllum*, which contain carvacrol as the main constituent, showed the greatest antimicrobial effect against MDR Salmonella strains.

## P060

# Inactivation of antibiotic-resistant microorganisms by physical plasma

Veronika Hahn[1], Martina Balazinski[1], Tilo Schulz[1], Michael Schmidt[1], Daniela Zühlke[2,3], Thomas von Woedtke[1,4], Jürgen Kolb[1], Klaus-Dieter Weltmann[1], Katharina Riedel[2,3]
[1]Leibniz Institute for Plasma Science and Technology (INP), Greifswald, Germany. [2]Institute of Marine Biotechnology, Greifswald, Germany. [3]Institute of Microbiology, University of Greifswald, Greifswald, Germany. [4]Institute for Hygiene and Environmental Medicine, Greifswald University Medicine, Greifswald, Germany

## Abstract

Wastewater treatment plants are „hotspots" for the dissemination of antibiotic-resistant microorganisms (ARM).[1] General treatment methods only insuffiently reduce the load.[2] Conversely, physical plasma methods have proven to be promising to inactivate microorganisms.[3]

Different plasma sources were tested according to their efficacy to inactivate ARM. Synthetic wastewater containing *Escherichia coli* GW-AmxH19 (isolate from hospital wastewater, Greifswald, Germany)[4] was treated with the respective plasma source for 30min. The viable count was determined before and after plasma treatment.

In dependence of the source a reduction of the viable count of 1-7 $\log_{10}$ was achieved.

Thus, physical plasma can be utilized as additional treatment stage in wastewater remediation and may also support the effort of „one health".

[1] Rizzo, L.; Manaia, C.; Merlin, C.; Schwartz, T.; Dagot, C.; Ploy, M. C.; Michael, I.; Fatta-Kassinos, D. *Science of the Total Environment* **2013,** *447*, 345-360.

[2] Prado, T.; Pereira, W. C.; Silva, D. M.; Seki, L. M.; Carvalho, A. P. D.; Asensi, M. D. *Letters in Applied Microbiology* **2008,** *46* (1), 136-141.

[3] Hahn, V.; Dikyol, C.; Altrock, B.; Schmidt, M.; Wende, K.; Ercan, U. K.; Weltmann, K. D.; von Woedtke, T. *Plasma Processes and Polymers* **2019,** *16* (5).

[4] Schneider, D.; Zühlke, D.; Petscheleit, T.; Poehlein, A.; Riedel, K.; Daniel, R. Germany. *Microbiology Resource Announcements* **2020,** *9* (21), e00279-20 (2pp).

## P061

# The diversity and occurrence of antibiotic resistance genes in the bovine, poultry, swine and soil environments.

Katie Lawther, Chris Creevey, Fernanda Godoy Santos, Linda Oyama, Sharon Huws
Queen's University Belfast, Belfast, United Kingdom

## Abstract

Antibiotic resistance poses a serious threat to human and animal health. The environmental resistome present in animal agriculture and soil may act as an antibiotic resistance gene reservoir, with the environmental microbiome being an origin of clinically relevant resistance genes.

This study describes the abundance and diversity of antibiotic resistance genes within bovine, poultry, swine and soil microbiomes. All available metagenomes within the NCBI Sequence Read Archive for the following microbiomes were assessed for antibiotic resistance genes: bovine gut/faeces, poultry gut/faeces, swine gut/faeces and soil. Blastn was used to search for 2,548 resistance genes, sourced from Resfinder, within 6,081 metagenomes. Blastn parameters included bitscore of >60, >99% percentage identity and the gene identified >25 times in a sequence. The metagenomic data was sourced from 55 countries and was >53million Mbases and >27Tb in size.

Across the metagenomes, 43,730 resistance genes were identified. Of the bovine, poultry, swine and soil samples analysed 95.1%, 99.3%, 92.2% and 93.9% respectively, contained at least one resistance gene. The number of different resistance genes identified in each microbiome was significantly different ($P$<.001). The poultry microbiome contained the highest average number of different resistance genes per sample (47.69), significantly higher than each microbiome ($P$<.001). Tetracycline resistance genes were the most prevalent in bovine and swine microbiomes, whereas macrolide resistance genes were most prevalent in poultry and soil. Resistance to last-resort drug colistin was found in poultry, swine and soil microbiomes. This study highlights the vast diversity of resistance within agricultural and soil microbiomes.

## P062

# Developing quantification methodologies for *Pseudomonas* biofilms

Lily Riordan, R. David Dunphy, Paul Murray, Christopher McCormick, Katherine R. Duncan
University of Strathclyde, Glasgow, United Kingdom

## Abstract

Antimicrobial resistance (AMR) is annually responsible for more than 700,000 deaths, however, is expected to increase to more than 10 million by 2050. Biofilm formation is one of the methods used by bacteria to afford themselves antibiotic resistance, reducing their growth rate and entering a dormant-like state; allowing them to withstand up to 1000x higher concentration of antibiotics than planktonic bacteria. This is a particular issue concerning AMR, as nearly all antibiotics target active metabolism. *Pseudomonas aeruginosa*, an opportunistic pathogen and prolific biofilm former, has shown resistance to nearly all classes of antibiotics, and is frequently responsible for implant and surgical site infections; forming biofilms on the inserted implant. However, discovery most often occurs after the implant has been explanted, or when the patient develops bacteraemia. In this work, we are developing biofilm detection methodologies to allow for accurate detection and quantification of biofilm forming bacteria. *P. aeruginosa* (PA14) biofilms were grown at a range of concentrations ($OD_{600}$0.05-1) and on medically relevant materials (316L stainless steel, polyester graft), and the ability of antibiotics and metabolite extracts from bacterial cultures to reduce biofilm formation was then tested. This was achieved through crystal violet staining and optical density measurements, as well as hyperspectral imaging (HSI) of *Pseudomonas putida* (KT2400) biofilms. These data showed reduced biofilm formation at sub-lethal antibiotic concentrations ($MIC_{50}$ = 3.125 µg/mL, concentration 50% biofilm reduction = 0.6 µg/mL), and the ability to quantify biofilm formation on medical implant materials and biofilms at different concentrations ($OD_{600}$0.05-1) using HSI.

## P063

# Transcriptomic analysis of the activity and mechanism of a ruthenium (II)-based antimicrobial that induces minimal evolution of pathogen resistance

Adam Varney, Samantha Mclean
Nottingham Trent University, Nottingham, United Kingdom

## Abstract

The World Health Organisation has declared antimicrobial resistance (AMR) as one of the top 10 global threats and estimates that 10 million annual deaths will be attributed to AMR by 2050. Di-nuclear ruthenium ($Ru1^{4+}$) offers a novel solution to the lack of new therapeutic antibiotics showing excellent antimicrobial potential against multi-drug resistance pathogens including *Escherichia coli* 958 (EC958).

Minimum inhibitory/bacterial concentration (MIC/MBC) assays were used to determine antimicrobial activity of ruthenium compounds. Time-kill assays were performed to investigate the effect of $Ru1^{4+}$ on actively growing cultures Evolution of resistance of EC958 against $Ru1^{4+}$ was established through daily passaging in media containing 0.5x-MIC $Ru1^{4+}$. qPCR was performed to probe the mechanism of action of $Ru1^{4+}$ targeting nine genes involved in DNA, membrane repair and other stress responses.

MIC assays demonstrate significant activity against several therapeutically relevant Gram negative pathogens with comparable values to standard antibiotics (2.9mg/L [1.6µM] against EC958). Evolution of resistance against $Ru1^{4+}$ was minimal in EC958; with four to eight-fold increases after five weeks of exposure. qPCR identified significant downregulation of *ompF* and *sdhA* genes. A significant increase in expression of *spy* a chaperone protein-coding gene occurred suggesting a requirement for repair of damaged proteins in the region of the outer membrane.

$Ru1^{4+}$ showed potent antimicrobial activity and slow evolution of resistance, which clearly demonstrates its potential as a novel antimicrobial, and this gene expression analysis in the presence of the compound lays the groundwork for a full transcriptomic study to fully explore the compound's mechanism of action.

## P064

# Redundancy of AcrA and AcrE to support efflux of antibiotics through AcrD in *Salmonella* Typhimurium

Ilyas Alav, Jessica Blair
University of Birmingham, Birmingham, United Kingdom

## Abstract

RND efflux pumps are important mediators of antibiotic resistance. RND efflux pumps including AcrB, are organized as tripartite systems, consisting of an inner membrane RND transporter, a periplasmic adaptor protein (PAP) and an outer membrane factor. We have previously identified the residues required for binding of the major PAP AcrA to the major RND pump AcrB and shown that there is promiscuity between the PAPs such that they can function with non-cognate pumps. AcrE is a PAP homologous to AcrA and AcrD and AcrF are RND pumps homologous to AcrB. This study aimed to determine whether the PAP AcrE can function with AcrD, which does not have its own PAP, to mediate efflux and whether the previously identified RND binding residues in AcrA and AcrE were also required for AcrD binding. The *acrD* and *acrE* genes were cloned into compatible vectors and co-transformed into a strain lacking *acrAB*, *acrD* and *acrEF*. When expressed together, *acrD* and *acrE* significantly decreased susceptibility of the efflux mutant strain to AcrD substrates including aztreonam, carbenicillin, cloxacillin, fusidic acid, nafcillin, novobiocin, oxacillin and ticarcillin, indicating that AcrE can also form a functional complex with AcrD. These experiments also highlighted that the substrate profile of AcrD in *S. enterica* and *E. coli* are different. Point mutations in the previously defined RND binding residues of AcrA and AcrE impaired AcrD-mediated efflux of substrate drugs which validates the interchangeability of AcrA and AcrE and highlights these residues as ideal drug targets for efflux inhibition to combat antimicrobial resistance.

## P065

# Investigating the Impact of Trace Elements on Antimicrobial Resistance of *Mycobacterium abscessus*

Yi Liu[1], Katy Murphy[1], Ivanna Pennisi[1], Ashleigh Cheyne[1], Rebekah Moore[1], Stanislav Strekopytov[2], Valeria Garbin[1], Mark Rehkämper[1], Gerald Larrouy-Maumus[1]
[1]Imperial College London, London, United Kingdom. [2]Natural History Museum, London, United Kingdom

## Abstract

*Mycobacterium abscessus*, a rapid-growing non-tuberculous mycobacterium that causes infections with increasing incidences, is termed as "clinical nightmare" due to difficulties in its treatment and its multiple resistance mechanisms to antibiotics. Trace elements are found widely in environment and has been shown to affect bacterial resistance to antibiotics. During my PhD I investigate the effects of inorganic ions on the drug susceptibility of *M. abscessus* and determine the underlying mechanisms of drug resistance using a combination of bioenergetics, metabolomics and RNA-sequencing. Particularly, I found that, as opposed to copper and cobalt, nickel affects drug tolerance of *M. abscessus* to various antibiotics including clarithromycin and amikacin. Treatment of clarithromycin in combination with nickel on *M. abscessus* resulted in increased drug uptake and membrane permeability, and bioenergetics studies using Seahorse extracellular flux analysis showed reduced TCA cycle activity. I further confirmed alterations in carbon and nitrogen metabolism by stable isotope labelling metabolic flux analysis. Further studies including RNA sequencing will be conducted to solidify my hypothesis of mechanism, that nickel affects metabolism of *M. abscessus* and make the bacteria enter a non-replicating state for the first stage of adaptation to that ion.

## P067

# Comparison of culture-based methods for the detection of AMR in surface waters

Leonie Henn
Edinburgh Napier University, Edinburgh, United Kingdom

## Abstract

The environment has been recognised as a factor in the spread of antimicrobial resistance (AMR). Surface waters contribute as they can be contaminated with resistant bacteria from sources such as sewage, domestic animals and wildlife. Furthermore, pollution with antibiotics or related contaminants can drive the development and spread of resistance genes. Exposure to AMR bacteria in these waters can occur directly though recreational activities and water supplies, or indirectly through contaminated food sources. This is a public health concern as infections with resistant bacteria become increasingly difficult to treat. This study compares different selective media and sampling protocols used for detecting and enumerating two AMR indicator organisms (ESBL resistant *E. coli* and vancomycin resistant *Enterococcus*) by membrane filtration. Seven samples were taken from the Water of Leith as either composite samples, consisting of three sub-samples taken at 30-minute intervals, or grab samples taken at a single time point. Samples were taken at 2 m distance to the bank and 20 cm depth. Three selective media were used for *E. coli*, MLGA, TBX and CCA, and were amended with 4 mg/L cefotaxime. Two selective media were used for enterococci, SB and ChromVRE agar, and were amended with 6 mg/L vancomycin. Total and resistant organisms were subsequently enumerated to compare selectivity and sensitivity of the media. Preliminary results suggest that there is no difference between grab and composite samples. While all media had a high selectivity, the sensitivity of CCA agar was slightly higher.

## P068

# Use of long read sequencing to characterise the genomic architecture of mobile genetic elements encoding *bla*$_{CTX\text{-}M\text{-}15}$ in *Escherichia coli* causing travellers' diarrhoea

Matthew Bird[1,2], Satheesh Nair[1], David Greig[1,3], Timothy Dallman[1,3], Saheer Gharbia[1,2], Claire Jenkins[1,3]
[1]Public Health England, London, United Kingdom. [2]NIHR Health Protection Research Unit, Warwick, United Kingdom. [3]NIHR Health Protection Research Unit, Liverpool, United Kingdom

## Abstract

Increasing levels of antimicrobial resistance (AMR) have been documented in *Escherichia coli* causing travellers' diarrhoea, particularly to the third-generation cephalosporins. Diarrhoeagenic *E. coli* (DEC) can act as a reservoir for the exchange of AMR genes between bacteria residing in the human gut, enabling them to survive and flourish through the selective pressures of antibiotic treatments.

Using Oxford Nanopore Technology (ONT), we sequenced eight different sequence types (ST) belonging to five different pathotypes of extended-beta-lactamase-producing DEC harbouring *bla*$_{CTX\text{-}M\text{-}15}$ from four patients recently returned to the UK from Pakistan. The aim of the study was to determine whether *bla*$_{CTX\text{-}M\text{-}15}$ was chromosome or plasmid-encoded to better understand the mechanisms of onward transmission of AMR determinants.

In Patient A, *bla*$_{CTX\text{-}M\text{-}15}$ was plasmid-encoded in both DEC isolates (ST504/ST3032), whereas in Patient B *bla*$_{CTX\text{-}M\text{-}15}$ was located on the chromosome in both DEC isolates (ST227/1283). Patients C and D both had one isolate where *bla*$_{CTX\text{-}M\text{-}15}$ was located on the plasmid and one chromosomally encoded (ST443/182 and ST38/99, respectively). The two plasmids associated with Patient A were different although one exhibited high similarity to the plasmid from Patient C. In the four isolates where *bla*$_{CTX\text{-}M\text{-}15}$ was chromosomally encoded, the site of insertion and the characteristics of the inserted plasmid segment differed.

Analysis of long-read sequencing data enables us to characterise the genomic architecture of mobile genetic elements encoding AMR determinants. These data may contribute to a better understanding of persistence and onward transmission of AMR determinants in MDR *E. coli* causing gastrointestinal and extra-intestinal infections.

## P069

# Comparison of genome-derived and phenotypic antimicrobial resistance profiles of *Shigella* species isolated from patients with symptoms of gastrointestinal disease in England, 2015-2020

Ching-Ying Poh[1], Amy Gentle[1], Claire Jenkins[1,2]
[1]Public Health England, London, United Kingdom. [2]NIHR Health Protection Unit, Liverpool, United Kingdom

## Abstract

Increasing antimicrobial resistance (AMR) in *Shigella* species is a global public health problem. We compared genotypic and phenotypic methods for the detection of AMR in *Shigella* species to evaluate the use of genome data for surveillance and monitoring of emerging AMR.

Whole genome sequencing (WGS) data from 388 isolates of all four *Shigella* species were analysed for the presence/absence of specific AMR determinants and selected accordingly. Phenotypic antimicrobial susceptibility testing was performed using in-agar dilution on all viable and pure isolates (n=358). The genotypic and phenotypic AMR profiles were then compared.

There were 335 (93.6%) isolates resistant to at least one antimicrobial and 222 (62%) isolates were multi-drug resistant, of which the majority (77%) were associated with foreign travel. Out of a possible 2864 isolate/antimicrobial class combinations, we identified 119 unexpected results, giving an overall concordance of 96.8% between the two methods. There were 54 samples that had an AMR determinant expected to confer resistance that were phenotypically susceptible, of which 31/54 (57.4%) were associated with tetracycline resistance and trimethoprim-sulfamethoxazole resistance. There were 65 that were phenotypically resistant to a specific antimicrobial class, but no AMR determinant was detected, of which 32/65 (49.2%) were associated with isolates harbouring a single *gyrA* mutation and exhibiting an unexpectedly high minimum inhibitory concentration (MIC) to ciprofloxacin.

Although comparisons between both methods showed good correlation between the genotypic and phenotypic AMR profiles, phenotypic monitoring is required to identify novel AMR mechanisms and to update reference database used for WGS analysis.

## P070

# Commensal *E. coli* are a reservoir for the transfer of XDR plasmids into epidemic fluoroquinolone-resistant *Shigella sonnei*

Duy Pham[1,2], To Nguyen[1], Duong Vu[1], Hao Chung[1], Felicity Alcock[1], Christine Boinett[1], Thanh Ho[1], Tuyen Ha[1], Guy Thwaites[1,2], Maia Rabaa[1,2], Stephen Baker[1,2]
[1]Oxford University Clinical Research Unit, Ho Chi Minh, Vietnam. [2]Centre for Tropical Medicine and Global Health, Oxford University, Oxford, United Kingdom

## Abstract

*Shigella spp*. are among the major causes of diarrhoeal disease globally. There are four *Shigella* species, but *Shigella sonnei* is becoming the most dominant internationally. The global dissemination of a ciprofloxacin-resistant (cipR) *S. sonnei* clone highlights the mobility of this organism and suggests that ciprofloxacin is no longer an appropriate first-line antimicrobial for shigellosis. Here, we track cipR *S. sonnei* after it entered Vietnam from South Asia to understand how novel antimicrobial resistant *Shigella* clones become established in new human populations. Between January 2014 and July 2016, we isolated and performed whole genome sequencing of 79 *S. sonnei* isolates from children hospitalized with dysentery in southern Vietnam to reconstruct the microevolution of cipR clone in this setting. We found that cipR *S. sonnei* displaced the resident ciprofloxacin-susceptible (cipS) lineage while rapidly acquiring additional resistance to multiple alternative antimicrobial classes. We identified several independent acquisitions of XDR/MDR-inducing plasmids, likely facilitated by horizontal transfer from commensals in the human gut. By characterizing commensal *E. coli* from Shigella-infected and healthy children, we identified an extensive array of AMR genes and plasmids, including an identical MDR plasmid isolated from both *S. sonnei* and *E. coli* in the gut of a single child. We additionally found that ciprofloxacin usage may impact plasmid transfer dynamics between commensal *E. coli* and *S. sonnei*. These results suggest that in a setting with high antimicrobial use and a high prevalence of AMR commensals, cipR *S. sonnei* may be propelled towards pan-resistance by adherence to outdated international treatment guidelines.

**P071**

# KPC-producing *Enterobacter cloacae* transfer through pipework between hospital sink waste traps in a laboratory model system

Paz Aranega Bou[1], Nicholas Ellaby[2], Matthew J. Ellington[2], Ginny Moore[1]
[1]Biosafety, Air and Water Microbiology Group, National Infection Service, Public Health England, Porton Down, United Kingdom. [2]National Infection Service, Public Health England, Colindale, United Kingdom

## Abstract

Carbapenemase-producing Enterobacterales (CPE) are an increasingly common cause of hospital-acquired infections whilst their reservoirs within the clinical setting remain poorly understood. Outbreaks have been linked to hospital sinks which can harbour and disperse CPE to surrounding surfaces. It has been proposed that Gram-negative organisms, including CPE, can migrate through plumbing biofilms.

Waste traps from a single hospital were installed in a laboratory model sink system incorporating six sinks connected through a common waste pipe. Drainage and tap flushing were automatically controlled. Nutrients were provided daily to maintain the bacterial populations which were regularly monitored. Three weeks after installation, waste traps were subjected to a drainage backflow event. Waste trap water populations continued to be monitored and when transfer between sinks was suspected, isolates were characterised and compared via whole-genome sequencing.

Immediately after a backflow event, two KPC-producing *E. cloacae* were recovered from a waste trap in which CPE had not been previously detected. The isolates were identified as ST501 and ST31 and were genetically indistinguishable to those colonising sinks elsewhere in the system. Following inter-sink transfer, KPC-producing *E. cloacae* ST501 successfully integrated into the microbiome of a recipient sink and was detected in the waste trap water for six months.

Sink waste traps and drains are a hospital reservoir for CPE. Once established, CPE contamination might not be confined to a single sink and could spread through wastewater plumbing. Potential mitigation strategies to reduce the risk to patients will also be discussed.

## P072

# Rapid detection and discrimination of closely related Enterobacteriaceae CTX-M group 1 variants, *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-15}$, using an internally controlled multiplex loop-primer endonuclease cleavage loop-mediated isothermal amplification (LEC-LAMP) assay

Owen Higgins[1], Alexandra Chueiri[1], Louise O'Connor[1], Liam Burke[1], Dearbhaile Morris[1], Marwa M. Hassan[2], Arnoud H. M. van Vliet[2], Roberto M. La Ragione[2], Nicola Maria Pfeifer[3], Belén González Santamarina[3], Christian Berens[3], Christian Menge[3], Terry Smith[1]
[1]National University of Ireland, Galway, Ireland. [2]University of Surrey, Guildford, United Kingdom. [3]Friedrich-Loeffler-Institut, Jena, Germany

## Abstract

Cefotaximases (CTX-Ms) are a class of plasmid-encoded extended-spectrum beta-lactamase (ESBL) enzymes found in Enterobacteriaceae such as *Escherichia coli* and *Klebsiella pneumoniae* that confer resistance to third-generation cephalosporin antibiotics. CTX-M enzymes are classified into five groups; CTX-M-1, 2, 8, 9 and 25. The rapid emergence and dissemination of CTX-M group 1 variants *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-15}$, typically associated with animal and human infection, respectively, is a global public-health concern and highlights the requirement for effective diagnostic tools. However, *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-15}$ variants are almost identical in nucleotide sequence and difficult to differentiate using conventional molecular diagnostics. Loop-primer endonuclease cleavage loop-mediated isothermal amplification (LEC-LAMP) is a recently developed technology that enables rapid real-time multiplex pathogen detection with single-base specificity and portable on-site testing applications. In this study we have developed an internally controlled multiplex LEC-LAMP assay for the differential detection of *bla*$_{CTX-M-1}$ and *bla*$_{CTX-M-15}$ variants in a single reaction. Analytical specificity and sensitivity of the *bla*$_{CTX-M-1/15}$ LEC-LAMP assay was established using clinical and environmental *E. coli* isolates from Ireland and Central Germany. The *bla*$_{CTX-M-1/15}$ LEC-LAMP assay demonstrated specific differential detection of both variants at high bacterial load concentrations of $10^6$ genome copies, and low-level detection for each variant of 10 genome copies per reaction in approximately 15-20 min. This assay will be further validated using *bla*$_{CTX-M}$ positive bovine and porcine faecal samples, and evaluated for on-site agricultural faecal sample testing in combination with portable instrumentation and a rapid bacterial DNA extraction protocol.

**P074**

# Whole Genome Sequencing of Multi-drug resistant *Pseudomonas* spp. isolated from bird faeces collected from recreational areas in Cambridge.

Joana Rodrigues[1], Harisree Paramel-Nair[1], Christopher O'Kane[1], Samuel J Bloomfield[2], Alison E Mather[2,3], Caray A Walker[1]
[1]Anglia Ruskin University, Cambridge, United Kingdom. [2]Quadram Institute Bioscience, Norwich, United Kingdom. [3]University of East Anglia, Norwich, United Kingdom

## Abstract

The rise in antimicrobial resistance (AMR) is of major concern to human and animal health globally. Surveillance within potential bacterial reservoirs is imperative. Drug resistant zoonotic bacteria, which have the ability to transfer from animals to humans, are of particular interest due to the potential public health risk. Spread can be a result of either direct or indirect contact. Many bacterial pathogens common to humans and domestic animals are also present in birds, including, but not limited to, *Pseudomonas* spp.. The incidence of *Pseudomonas* spp. bacteraemia has been increasing with 8.1 reports per 100,000 population in England, Wales and Northern Ireland. Here, we evaluated wild birds as a potential reservoir of AMR and *Pseudomonas* spp. Wild bird faecal samples were collected from recreational areas around the river Cam. This study area was incorporated due to the habitat which is popular with wild birds (including corvids, gulls and water fowl) as well as humans. Microbiological and molecular techniques were used to isolate *Pseudomonas* spp. prior to standard Kirby Bauer testing using meropenem, cefepime, gentamicin, ciprofloxacin and levofloxacin. This was followed up with whole genome sequencing to determine the genetic AMR mechanisms. We collected *ca.* 115 faecal swabs, of which 24 (20.9%) were positive for *Pseudomonas* spp. A total of 83% and 88% Pseudomonads were resistant to fluoroquinolone and cephalosporin, respectively. Some isolates also displayed resistance to gentamicin. This work confirms that wild birds carry bacteria resistant to antimicrobials important for human health and may act as a potential reservoir of AMR bacteria.

## P075

# Genotypic and phenotypic patterns of antimicrobial susceptibility of anaerobes isolated from healthy people in Vietnam and Japan

Hanh Vu[1], Yoshimasa Yamamoto[2], Masahiro Hayashi[2], Kaori Tanaka[1]
[1]United Graduate School of Drug Discovery and Medical Information Sciences - Gifu University, Gifu, Japan. [2]Life Science Research Center - Gifu University, Gifu, Japan

### Abstract

Normal non-pathogenic flora could be harming the host by acting as a reservoir of resistance determinants potentially transferable to human pathogens. To assess the phenotypic and genotypic antimicrobial susceptibility patterns of anaerobes isolated from normal flora of Vietnamese and Japanese people, 199 anaerobes were obtained from the faecal samples of 80 healthy individuals (51 Vietnamese and 29 Japanese). The identification of isolated strains was done using MALDI-TOF MS. The minimum inhibitory concentration (MIC) values were determined using an agar dilution method. Nitrocefin discs were used to detect the production of β-lactamase. The presence of the resistance genes (cepA, cfxA, cfiA, nim, ermB, ermF, ermG, linA, mefA, msrSA, tetM, tetQ, tetX, tetX1, tet36, bexA, qnrA, qnrB, qnrS and catA) and one virulence gene (bft) was determined by standard PCR. The prevalence of resistance genes was compared with the phenotypic resistance and between countries. Diversity in the phenotypic and genotypic antimicrobial susceptibility patterns in the same species isolated from the same person also found in many cases. The high prevalence of antibiotic-resistant genes related to plasmids and conjugative transposons showed the highly transferable ability of those genes to other human pathogens. This study is the first report on the antimicrobial susceptibility patterns in anaerobes isolated from healthy people in Vietnam and Japan. It also accessed the highest number of antibiotics and its resistance genes in the field of anaerobic research so far. Further investigations will be carried to show the evidence and mechanism of transmission of resistance determinants to human pathogens.

## P076

# Antimicrobial resistance: revisiting the mechanisms of resistance

Ifeanyi Elibe Mba, Onyekachi Philomena Okeke, Hyelnaya Cletus Sharndama, Goodness Ogechi Osonduchuka, Joseph Ukuomadu, Chidera Ugwu
University of Nigeria Nsukka, Nsukka, Nigeria

## Abstract

Antimicrobial resistance: revisiting the mechanisms of resistance

Abstract

Resistance to antibiotics persists as a critical challenge in public health. Currently, the emergence of multi-drug resistant (MDR) bacteria is a primary concern globally, resulting in a dramatic increase in epidemiological relevance and importance of nosocomial and chronic infections. Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacteriaceae has recently been classified as critical in the World Health Organization (WHO) priority pathogens. Among these bacterial pathogens, resistance seems to be a natural trait. The acquisition and development of resistance by bacteria is through several mechanisms. The genetic background and intrinsic resistance mechanisms largely contribute to competitive advantage and resistance in a highly resistant pool. The acquisition of resistance genes driven by mobile genetic elements (MGE) and several biochemical mechanisms also plays a central role in resistance development among pathogenic bacteria. This review discussed the recent underlying multiple resistance mechanisms among the priority pathogens. This review also provides an up-to-date regional epidemiological data and implications of antimicrobial resistance. Given the severity of infections caused by these bacteria, their less susceptibility to the available antimicrobials, and the limited antimicrobial arsenal to treat these pathogens, current insight on resistance mechanisms becomes timely and highly relevant. This information will help develop better therapeutic strategies against resistance microbes, especially those of urgent priority.

Keywords: Antimicrobials, multi-drug resistance (MDR) bacteria, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacteriaceae

## P077

# Investigating copper hyper-resistance in *Staphylococcus aureus* USA300

Daniella Spencer[1], Joanne Purves[1], Inderpreet Kaur[1], Joan Geoghegan[2], Kevin Waldron[3], Peter Andrew[1], Julie Morrissey[1]
[1]Leicester University, Leicester, United Kingdom. [2]Birmingham University, Birmingham, United Kingdom. [3]Newcastle University, Newcastle, United Kingdom

## Abstract

Community acquired, methicillin resistant *Staphylococcus aureus* (CA-MRSA) is on the rise. Transmitting from person to person outside the healthcare environment, CA-MRSA clones can be more virulent and show increased tolerance to unfavourable conditions. The USA300 CA-MRSA strain is already endemic in the United States. USA300 demonstrates a heightened resistance to the innate immune system, particularly to macrophage engulfment. Two horizontally acquired genes, encoding an efflux pump (CopX) and lipoprotein (CopL), were discovered in 2 different lineages of USA300, representing CA-MRSA epidemics in North and South America. Removal of either gene resulted in elevated cytoplasmic copper concentrations in *S. aureus*, implying a function in copper hyper-resistance. While copper is an essential part of metabolic machinery, it is toxic at high concentrations and is utilised by macrophages to kill bacteria in the phagosome. Supporting this, USA300 with functional *copXL* genes showed increased survival in macrophages compared to their *copXL* negative counterparts. Although the role of CopX as an efflux pump explains the rise in intracellular copper concentration upon its mutation, the role of the CopL lipoprotein is still unknown. Therefore, to better understand the function of CopL and how it might influence *S. aureus* host interaction, RNAseq was utilised to identify divergent patterns of gene expression between WT and *copL* mutant strains grown in the presence of copper. This has led to a greater understanding of the mechanisms of copper toxicity and how USA300 has adapted to overcome the associated disruption to cellular function, contributing to its success as an environmental and pathogenic organism.

## P078

# *Escherichia coli* ST11 (O157:H7) does not have a functional AcrF efflux pump

Hannah L. Pugh, Christopher Connor, Alan McNally, Jessica M. A. Blair
University of Birmingham, Birmingham, United Kingdom

## Abstract

Resistance nodulation division (RND) efflux pumps are tripartite complexes that span the cell membrane and cell wall and mediate antibiotic resistance. The *E. coli* type strain MG1655 has six RND pumps, *acrB, acrD, acrF, mdtBC, mdtF* and *cusA.* It has been assumed that all *E. coli* lineages encode the same efflux systems and with identical sequences. This work set out to test this assumption, focusing on the two pumps most commonly associated with MDR and virulence, AcrAB and AcrEF.

Over 18000 genome assemblies from 18 *E. coli* sequence types (STs) were downloaded from Enterobase and used to construct pangenomes using Roary. With the sequences for *acrAB* and *acrEF* used as the references, RND alleles were determined (groups of sequences with ≥95% sequence identity to the reference).

Both efflux systems were highly conserved however, *acrEF* was more varied than *acrAB*. The most variable gene was *acrF* which is a close homolog of *acrB.* However, no alleles were identified in ST11, a human pathogen with virulence factors that include the Shiga-toxin. This was found to be due to a highly conserved insertion of 15 amino acids, two of which encode stop codons, in the *acrF* gene resulting in truncation. The truncated AcrF of ST11 was shown experimentally to be non-functional.

This work implies that the number of RND pumps found across different isolates of a single species are not consistent. Conservation may be lineage, and subsequently, lifestyle dependent.

## P079

# Investigating Selection and Co-selection for Antimicrobial Resistance by Plant Protection Products (PPPs)

Laura Murray[1], Lihong Zhang[1], Alistair Boxall[2], Jason Snape[3], William Gaze[1], Aimee Murray[1]
[1]University of Exeter, Cornwall, United Kingdom. [2]University of York, York, United Kingdom.
[3]AstraZeneca, Macclesfield, United Kingdom

## Abstract

Antibiotic and non-antibiotic plant protection products (PPPs) are applied at high concentrations and quantities, directly to crops and soils during agricultural processes. They are applied alongside manures that may contain antibiotic residues, other selective agents and resistant bacteria. Selection pressures exerted by these chemicals may result in enrichment of resistant bacteria and/or the exchange of resistance genes between environmental and clinically relevant bacteria. Presently, there is little research looking at the effects of PPPs on the development and spread of antibiotic resistance. As a result, the impact of these chemicals on resistance and microbial diversity, particularly in terrestrial environments is poorly understood.

Using the SELECT method recently developed by Murray *et al.,* (2020), the antibiotics kasugamycin (used only in crop agriculture), streptomycin and gentamycin (used in both medicine and crop agriculture) were tested with mixed microbial communities. Selective concentrations were identified by a significant reduction in community growth. Results from these experiments informed the concentrations at which long term evolution experiments were carried out. Selection for resistance was investigated using targeted real-time PCR and metagenomic analysis.

Results from these experiments will provide information crop antibiotics and their effects on environmental antibiotic resistance (ABR). This understanding is of great importance, particularly with regards to human exposure to environmental reservoirs of resistant bacteria.

## P080

# The role of staphylococcal bacteriocins in nasal competition

Joshua Thomas, Melanie Ghoul, Daniel Wilson, Ashleigh Griffin
University of Oxford, Oxford, United Kingdom

## Abstract

Bacteriocins are antimicrobial toxins produced by bacteria to defend and invade territories by killing unrelated strains and species. Understanding if bacteriocins shape natural populations is important for understanding the evolution of antimicrobial resistance and identifying novel antimicrobials for clinical use. *Staphylococcus aureus* is an opportunistic pathogen that asymptomatically colonises the nasal cavity of 1 in 3 healthy adults. *S. aureus* is known to produce many different bacteriocins, however we are yet to understand the extent to which they mediate the establishment of nasal populations. Here, we test the importance of bacteriocins in driving colonisation success, by screening *S. aureus* antimicrobial inhibition against other *S. aureus* strains and three commensal species that commonly co-inhabit the nasal cavity. We use a longitudinally sampled collection of 173 *S. aureus* nasal isolates from 14 participants over 90-months to track within-individual population changes over time. We found that 8% of all *S. aureus* isolates produced bacteriocins active against other species, but that between-strain bacteriocin inhibition in *S. aureus* was very rare, observed by only 0.5% of isolates. Therefore, while there is no evidence that intraspecific competition drives colonisation success in the nasal cavity, interspecific competition is more likely to influence strain and species dominance.

## P082

# Antibiotic resistance in Antarctica: no longer a pristine ecosystem?

Kudzai Hwengwere[1,2], Dr Caray Walker[1], Professor Melody Clark[2], Dr Harisree Paramel-Nair[1]
[1]Anglia Ruskin University, Cambridge, United Kingdom. [2]British Antarctic Survey, Cambridge, United Kingdom

## Abstract

Although Antarctica is considered the last pristine continent, human activity (via research bases, tourism, and expeditions) and environmental factors may be working synergistically to promote the spread of human-associated bacteria and antibiotic resistance genes (ARGs) in this region. The global issue of antibiotic resistance necessitates more systematic surveys to better understand the factors involved in the spread of ARGs. Mapping the transmission of ARGs in pristine environments such as Antarctica, where AMR levels are low, can inform on selective pressures and barriers to the spread of antibiotic resistance and shed light on the global spread of ARGs. In addition, knowledge of the extent of AMR in Antarctica will contribute to future, long-term strategies and environmental policy in this unique region. In this poster, we present our latest data screening for the presence of ARGs in animal scat and environmental samples taken from Antarctica, using culture-based and bioinformatics approaches. Results will be compared with published studies on AMR in Antarctica and ARG screening of published Antarctic metagenomic environmental samples, using bioinformatic tools such as ResFinder and AMRFinder.

## P083

# Synergistic -cidal effect of antimicrobial peptides with chitosan nanosystems against multi drug resistant *Acinetobacter baumannii*

Afreenish Hassan, Aamer Ikram
NUMS, Rawalpindi, Pakistan

## Abstract

*Acinetobacter baumannii* causes critical drug resistant nosocomial infections in immunocompromised patients. There is need to develop therapeutic solutions that can effectively address resistance crisis. Nanoparticles and antimicrobial peptides have emerged as upcoming strategy, when methodically applied together, can offer robust bactericidal effect against multidrug resistant (MDR) bacteria.

Chitosan-mastoparan nanosystem is prepared by ionic gelation method, and nanoparticles are characterized by dynamic light scattering, scanning electron microscopy and fourier transform infrared spectroscopy. In vitro, broth microdilution of chitosan-mastoparan nanosystem is performed to estimate minimum inhibitory concentration against MDR *A. baumannii* clinical isolates.

The in vitro synthesis optimized for chitosan:sodium triployphosphate ratio, pH, temperature yields ~85-~101 nm mastoparan loaded chitosan nanoparticles, zeta potential ~55 mV with loading capacity 22.6% and encapsulation efficiency 90.5%.

Antibacterial experiments demonstrated that lowest concentration (MIC90) inhibiting growth of MDR *A. baumannii* for chitosan-mastoparan nanosystem is 4µg/ml, while MIC90 value was higher than 256ug/ml for chitosan (control). Significant statistical difference is found between MIC values for chitosan-mastoparan nanosystem: chitosan alone and chitosan-peptide nanosystemt:mastoparan alone (p value <0.05).

Chitosan-mastoparan nanosystem provided synergistic bactericidal effect against MDR *A. baumannii* clinical isolates at low concentrations. It can serve as alternative promising treatment option against rapidly prevailing multi drug resistant *A. baumannii* strains.

## P084

# Phenotypic whole-cell screening identifies a protective carbohydrate epitope on *Klebsiella pneumoniae*

Sophia Berry[1,2], Carolina Caceres[3], Steven Rust[2], Ralph Minter[2], Andrew Grant[1]
[1]University of Cambrige, Cambridge, United Kingdom. [2]AstraZeneca, Cambridge, United Kingdom.
[3]AstraZeneca, Gaithersburg, USA

## Abstract

The increasing occurrence of recalcitrant multi-drug resistant (MDR) *Klebsiella pneumoniae* infections coupled with a diminishing pipeline of new antibiotics warrants the investigation of alternative antimicrobial therapies. We employed a target-agnostic phage display approach using live *K. pneumoniae* bacteria with the aim of isolating therapeutic monoclonal antibodies (mAbs) targeting conserved epitopes among clinically relevant strains. mAb targets were explored using ELISA and biolayer interferometry, and a high-throughput opsonophagocytic killing assay was developed to determine functional activity. Fluorescence-activated cell sorting was used to screen a global panel of clinical isolates, and high-content imaging further explored binding and functional activity. One mAb was tested *in vivo* using a lethal murine model of pneumonia. mAbs binding to carbohydrate epitopes were isolated in phage display selections enriched on wild-type and capsule-deficient strains. mAbs binding O1 lipopolysaccharide (LPS) and cross-binding O1/O2 LPS were identified. mAbs were shown to promote opsonophagocytic killing by human monocyte-derived macrophages, and clearance of macrophage-associated bacteria. One mAb, named B39, protected mice against MDR O1 and O2 strains when dosed therapeutically in a murine pneumonia model. Binding to a panel of O1 and O2 clinical isolates suggests B39 binds to both D-galactan-I and D-galactan-II of the LPS. With the rise of antimicrobial resistance among enteric pathogens, the discovery of a novel therapeutic mAb targeting the most prevalent *K. pneumoniae* serotypes demonstrates a significant advancement in the field, and showcases the potential of alternative antimicrobial therapies for the treatment of MDR infections.

## P085

# Identification of novel *Pseudomonas aeruginosa* small non-coding RNAs and their role in biofilm and antibiotic resistance mechanisms

Beatriz Carvalho, Cecília M. Arraiano, Vânia Pobre
ITQB, Oeiras, Portugal

## Abstract

The irresponsible use of antibiotics is triggering the emergence of antimicrobial resistant (AMR) bacterial strains. Currently, at least 700,000 people die each year due to drug-resistant diseases. The World Health Organization identified *Pseudomonas aeruginosa* as one of the three most critical bacterial pathogens for which novel therapeutical options are in urgent need. This opportunistic pathogen poses a serious health threat to hospitals and other healthcare settings causing a wide range of infections. Beyond its natural resistance to many drugs, this bacterium makes biofilms as a protection against immune defence systems and antibiotherapy.

Small non-coding RNAs (sRNAs) play a key role in controlling gene expression. A recent study has shown that the overexpression of one single sRNA led to an increase in drug susceptibility in *P. aeruginosa*. However, our current knowledge of sRNAs in this organism is very limited. In this work we used public RNA-Seq data from *P. aeruginosa* PAO1 biofilm culture and planktonic cells grown in catheters without antibiotic coating or with clindamycin/rifampicin coating. With these datasets, we predicted novel sRNAs using two different programs (sRNA detect and ARTEMIS) to identify putative sRNA regions with high expression values. We found 1083 novel sRNAs that had high expression in at least one condition. We have also discovered that there is a cluster of sRNAs that are highly expressed in planktonic cells when subjected to the antibiotics. The identification of these novel sRNAs is of great importance since it provides possible new targets to fight *P. aeruginosa* strains resistant to antibiotics.

## P086

# The detection of duplicated metabolic genes in S. epidermidis isolates that confer decrease susceptibility to AMD

Nada Almebairik[1,2], Adam Roberts[3], Julie Morrissey[1], Karolin Hijazi[4], Marco Oggioni[1]
[1]University of Leicester, Leicester, United Kingdom. [2]King Saud University, Riyadh, Saudi Arabia. [3]2Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [4]3University of Aberdeen, Aberdeen, United Kingdom

## Abstract

The high capacity of bacteria to resist antimicrobial compounds is due to the rapid horizontal spread of mobile genetic elements that carry antimicrobial resistance (AMR) genes. Often these mobilised genes are metabolic genes, but upon transfer to another organism, the duplicated metabolic core gene may confer reduced susceptibility to antibiotics and biocides. While much effort is placed in tracing known resistance genes, there is scarce work on their metabolic origin, therefore we aimed in this study to discover the duplicated metabolic gene in *Staphylococcus epidermidis* core-genome.

The genomic DNA of 58 *S. epidermidis* clinical isolates, collected between 2009 and 2018 in Aberdeen, was prepared followed by whole genome sequencing. Core and accessory genomes for *S. epidermidis* have been identified using Roary software. Several genomic analysis have been used to create a list of duplicated core genes using *S. epidermidis* ATCC12228 as a reference. Three out of four ST5 *S. epidermidis* isolates were found to harbour 5 duplicated core genes in 2 operons that found to involved in the pentose phosphate pathway and formaldehyde assimilation. The three ST5 isolates with the duplicated genes show increase of the formaldehyde MIC by one dilution compared to other isolates. In addition, one isolate was found to carry a 2$^{nd}$ copy of ohyA oleate hydratase gene and this isolate shows increase of the antimicrobial fatty acid MIC compared to other isolates. This is the first report showing that duplication of the ribulose monophosphate pathway confers reduced susceptibility to formaldehyde to staphylococci.

## P087

# Potential for prodrug therapy to overcome antibiotic tolerance of biofilms

Rikke Louise Meyer, Raoul Walther, Signe Maria Nielsen, Rikke Christiansen, Alexander Zelikin
Aarhus University, Aarhus, Denmark

## Abstract

Antibiotic treatment of biofilm infections often fail because the dose that can be administered safely is insufficient to eradicate the infection due to the high antibiotic tolerance of biofilms. We hypothesize that treatment will be more effective if drugs are delivered or synthesized at the site of infection, leading to a high local concentration with minimal side effects. Conventional drug delivery using encapsulated drugs with a burst release offers little control over dosage and exposure time. We therefore aimed at delivering a high local dose of antibiotics through local drug synthesis through prodrug therapy.

Prodrug therapy uses immobilized enzymes to convert non-toxic prodrugs to the active drug. We developed a novel method for synthesizing glucuronide prodrugs, opening the door for antimicrobial prodrug therapy. We immobilized the catalyst (β-glucuronidase) in a layer-by-layer coating on titanium implants and investigated the effect of moxifloxacin glucuronide prodrugs on *S. aureus* viability and biofilm formation.

The embedded enzyme continuously converted the inactive prodrug to moxifloxacin at the implant surface, which prevented biofilm formation, even under flow. Prodrug therapy offers flexibility for exposure time and drug concentration, and we believe that our encouraging results will pave the way for implementing more potent drugs that target persister cells in treatment of biofilm infections.

## P088

# Systematic Reconstruction of the Complete Two-Component Sensorial Network in Staphylococcus aureus

Andreas Haag[1], Beatriz Rapun-Araiz[2], Virginia De Cesare[3], Pedro Dorado-Morales[2], José Penadés[1,4,5], Iñigo Lasa[2]
[1]Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom. [2]Laboratory of Microbial Pathogenesis, Navarrabiomed, Complejo Hospitalario de Navarra (CHN)-Universidad Pública de Navarra (UPNA), IDISNA, Pamplona, Spain. [3]MRC Protein Phosphorylation and Ubiquitylation Unit, University of Dundee, Dundee, United Kingdom. [4]Departamento de Ciencias Biomédicas, Universidad CEU Cardenal Herrera, Moncada, Spain. [5]MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, United Kingdom

## Abstract

In bacteria, adaptation to changes in the environment is mainly controlled through two-component signal transduction systems (TCSs). Most bacteria contain dozens of TCSs, each of them responsible for sensing a different range of signals and controlling the expression of a repertoire of target genes (regulon). Frequently, TCS control key physiological changes required for pathogenesis and/or antimicrobial resistance. Over the years, identification of the regulon controlled by each individual TCS in different bacteria has been a recurrent question. However, limitations associated with the classical approaches used have left our knowledge far from complete. In this report, using a pioneering approach in which a strain devoid of the complete nonessential TCS network was systematically complemented with the constitutively active form of each response regulator, we have reconstituted the regulon of each TCS of *S. aureus* in the absence of interference between members of the family. Transcriptome sequencing (RNA-Seq) and proteomics allowed us to determine the size, complexity, and insulation of each regulon and to identify the genes regulated exclusively by one or many TCSs. This gain-of-function strategy provides the first description of the complete TCS regulon in a living cell, which we expect will be useful to understand the pathobiology of this important pathogen.

## P089

# A Systematic review of animal production practices and how they contribute to AMR in the environment.

Jennifer Brazier
University of Nottingham, Nottingham, United Kingdom

## Abstract

Background: AMR contamination of the farming environment is a potential exposure route for AMR infections in humans. Understanding the role that animal production practices play in contributing to the Resistome is required to help quantify the risk and help develop possible interventions and production animal management changes to reduce the risk of AMR across human and veterinary contexts.

Methods: A Systematic review was undertaken of the published literature. PubMed and Google Scholar databases were searched. Title, abstract and full paper reviews were undertaken to determine papers with changes in AMR in the environment after intervention of animal production factors. Reference list searching of papers included in full paper analysis was conducted and the same inclusion criteria applied.

Results: Key animal production factors include slurry/waste application to farmland containing antimicrobial residues, AMR bacteria and ARGs. Animal faeces contamination of crop farmland. Rain runoff from farmland, direct application of antibiotics to aquaculture environments and even aerosol transmission. These provide multiple exposure routes to humans through direct contact and contamination of non-animal food products, which are often eaten raw.

Conclusions: Current animal production practices have a direct effect on the environmental Resistome and are a key area to investigate to mitigate the overall risk of AMR for human and animal health.

## P090

# A paradox of bacterial persistence and antibiotic resistance: chloramphenicol acetyl transferase as a double barrel shot gun

Ana Alves da Silva, Inês Jesus Silva, Cecília Maria Arraiano
Instituto de Tecnologia Quimica e Biológica - Universidade NOVA de Lisboa, Oeiras, Portugal

## Abstract

The growing concern about antimicrobial resistance has led to an increasing interest in bacterial persistence and its impact on infection in the host. Nonetheless, these two mechanisms are often assessed in independent studies and there is a lack of knowledge about their relation or possible interactions between them, both at cell and population level. Our work shows evidence that the insertion of the resistance gene Chloramphenicol Acetyl Transferase (*cat*) together with its cognate antibiotic chloramphenicol (CAM), an inhibitor of protein synthesis, is capable to modulate *Salmonella* Typhimurium persistence to several antibiotics by decreasing its level of survival in time. Recently, it was proposed that RelA ((p)ppGpp synthetase) binds to uncharged tRNAs, forming RelA.tRNA complexes that bind to vacant A-sites in the ribosome, being this mechanism essential for RelA activation. We propose that the antibiotic chloramphenicol blocks the A-site of the ribosome, hindering the RelA.tRNA complexes to bind to the ribosome thus preventing the activation of RelA and (p)ppGpp synthesis decreasing the level of persistence of the population.

## P092

# Combining sewage- and hospital- based surveillance data for fluoroquinolone resistance abundance in Europe: positive associations with antimicrobial usage rates

Hannah Lepper, Mark Woolhouse, Bram van Bunnik
University of Edinburgh, Edinburgh, United Kingdom

### Abstract

Background:

More needs to be known about AMR in the community, but most surveillance data is hospital-based and the relationship between resistance in hospitals and the community is not well understood. This study combines phenotypic hospital-based surveillance of fluoroquinolone resistance with municipal wastewater metagenomics data on fluoroquinolone resistance gene abundance to investigate how and why hospital and community resistance may correlate.

Methods:

We use data from the Global Sewage Surveillance Project and EARS-Net (ECDC) to obtain country level correlations between hospital and wastewater data with and without antimicrobial usage (AMU) rates, and coefficients for association between these resistance measures and usage rates. We use a multivariate mixed effects model that links a Poisson model of resistance genes and a binomial model of isolate resistance through country-level covariance.

Results:

For fluoroquinolones, community usage and resistance gene abundance were correlated (rate ratio 1.4, 95% confidence intervals 1.0-2.1), as were hospital usage and the proportion of hospital isolates that were resistant, except for *E. coli* (*Acinetobacter* spp.: odds ratio 212.3, 68.7 – 735.6; *E. coli*: 1.8, 0.8 – 4.3; *K. pneumoniae*: 3.3, 1.3 - 8.1; *P. aeruginosa*: 5.0, 1.8 - 13.0). Country-level correlation between the two datasets was 0.25 (-0.3-0.8) with AMU, but 0.6 (0.2-0.8) without.

Conclusion:

Hospital and community-based fluoroquinolone usage are key factors in fluoroquinolone resistance. Resistomes in hospitals and communities may be similar due to shared antimicrobial usage patterns, or because usage in the one directly affects resistance rates in the other.

## P093

# Does our microbiome travel well? Microbiome resilience and acquisition of multidrug resistant bacteria in travellers

Matthew Davies[1,2], Willem van Schaik[2], Alan McNally[2], Petra Wolffs[3], John Penders[1,3]
[1]Maastricht University, Maastricht, Netherlands. [2]University of Birmingham, Birmingham, United Kingdom. [3]Maastricht University Medical Centre, Maastricht, Netherlands

### Abstract

The international spread of antimicrobial resistance poses a serious health risk, as many travellers visit countries that are hotspots of resistance. A previous study focussing on the carriage of multidrug resistant bacteria after travel showed that there is extensive acquisition and persistence of extended spectrum beta lactamase producing Enterobacteriaceae (ESBL-E) in the gut of travellers visiting Asia and Africa. Using shotgun sequencing data from 190 of these travellers, the metagenomics profile of the gut microbiome has been analysed to understand its role in this context.

A metagenomics approach was used to determine the taxonomic composition and population diversity at baseline (before travel) and how these are altered longitudinally. Predicted genes are clustered by their abundance profile across multiple samples, providing a more powerful signal for analysing metagenome data. Here we show that these aspects at baseline do not significantly differ between travellers that were or were not subsequently colonised by ESBL-E, so are not predictive of the risk of acquiring ESBLs. However, there were longitudinal changes detected in the taxonomy which were specific to the travel destination.

The lack in predictive power of the baseline microbiome suggests that a traveller's risk of ESBL acquisition is difficult to determine before travel. Alternatively, the longitudinal results highlight the consortia of bacteria that may have a role in the protection against, or clearance of, ESBL producing Enterobacteriaceae. These are therefore potential targets as a prophylactic treatment or as adjuvants in the decolonisation of ESBL-E.

## P094

# Identification and characterization of putative bacteriocin-producing coagulasenegative Staphylococci (CoNS)

Maija Kisite, Laura Sheehy, Caitríona Guinane, Máire Begley
Munster Technological University, Cork, Ireland

## Abstract

A rise in bacterial resistance to conventional antibiotics means alternative control strategies are urgently required. Natural antimicrobials such as bacteriocins (microbially produced antimicrobial peptides) have received much interest in this regard. The aim of the present study was to assemble a bank of CoNS and examine them for their ability to produce potential novel bacteriocins. 40 CoNS were isolated from the skin of 20 human volunteers by swabbing the right nasal vestibule and the cubital fossa and plating onto mannitol salt agar. The strains were assessed for antimicrobial activity using an agar-based deferred antagonism assay, 95% (38/40) of the isolates inhibited the indicator microorganism *Micrococcus luteus*.

Twenty four isolates were identified as *Staphylococcus epidermidis* (67%), *Staphylococcus hominis* (17%), *Staphylococcus capitis* (8%), *Staphylococcus warneri* (4%) and *Staphylococcus lugdunensis* (4%) using a combination of 16S PCRs and API Staph. A particular strain of interest *Staphylococcus epidermidis* (C15) showed that the antimicrobial substance, in a well diffusion assay, is secreted into the supernatant. Proteinase-K assay revealed that from the twenty-four shortlisted strains, five strains produced an antimicrobial substance whose activity was reduced, indicating they are proteinaceous in nature. Genome sequencing of selected CoNS strains are underway and future experiments include peptide purification. The ability of a bacteriocin to target specific pathogens of similar phenotype, as the producer makes them a good candidate in helping combat the rise in multidrug-resistant pathogenic bacteria and treat CoNS-related infections.

## P095

# TcaA: not only glycopeptide resistance

Edward Douglas[1], Tarcisio Brignoli[1], Marieangela Wilson[1], Eoin O'Brian[2], Fernando Ponce Garcia[1], Borko Amulic[1], Mario Recker[3], Rachel McLoughlin[2], Ruth Massey[1]
[1]University of Bristol, Bristol, United Kingdom. [2]Trinity College, Dublin, Ireland. [3]University of Exeter, Exeter, United Kingdom

## Abstract

*Staphylococcus aureus* is an opportunistic human pathogen with an impressive array of virulence factors, some of which contribute to the evasion from the host immune system. A genome wide association study using 300 bacteraemia isolates identified a single nucleotide polymorphism (SNP) associated with an increase in serum resistance. The SNP was found to be in the *tcaA* gene, a member of the cell-wall stimulon. TcaA was originally described as a glycopeptide resistance determinant, whereby loss of the *tca* operon was associated with reduced susceptibility to teicoplanin and vancomycin through an unidentified mechanism. Here we show that a *tcaA* mutant is better able to survive host defence fatty acids (HDFAs) and cationic antimicrobial peptides (CAMPs) indicating a role in immune evasion. Moreover, we demonstrate that the *tcaA* mutant has altered cell-wall biosynthesis, characterised by reduced crosslinking and increased wall teichoic acid (WTA). WTA abundance and modifications are central to the ability of *S. aureus* to evade the killing action of HDFAs and CAMPs. Alterations in the cell surface charge due to the incorporation of d-alanine in WTA can provide an electrostatic repulsion, preventing the access of CAMPs to the bacterial membrane. Here we show that the *tcaA* mutant has a relative increase in surface positivity due to a transcriptional reduction in FmtA. In conclusion, our data show that TcaA influences cell-wall architecture with clear effects on glycopeptide and serum resistance. Additionally, the loss of *tcaA* expression appears to be an important step for *S. aureus* bacteraemia pathogenicity.

## P096

# Vegetables and rhizospheres as the reservoir for extensively drug-resistant (XDR) *Pseudomonas aeruginosa*

Ambreetha Sakthivel[1,2], Kalai Mathee[2], Balachandar Dananjeyan[1]
[1]Tamil Nadu Agricultural University, Coimbatore, India. [2]Florida International University, Miami, USA

## Abstract

*Pseudomonas aeruginosa* is an urgent threat pathogen due to its evolving resistance to multiple antibiotics. Agricultural soil and plants are the vast reservoirs of this much-dreaded opportunistic bacterium. A few human isolates of *P. aeruginosa* are known to infect plants and insects. However, there is no report on the occurrence of multi-drug resistant *P. aeruginosa* in edible vegetable crops. This study compared 18 *P. aeruginosa* isolates from the rhizosphere and endophytic niches of four different vegetable crops (cucumber, tomato, eggplant, and chili) with three known clinical strains. All the isolates were tested for virulence traits such as resistance to various antibiotics classes, motility, biofilm, and production of virulence factors (rhamnolipid, pyocyanin, hemolysin, proteases, and lipases). Hierarchical clustering based on Ward minimum variance with Manhattan distance matrix grouped the strains into three clusters based on their phenotypic traits. Strains were exhibiting the highest virulence co-clustered with the human pathogenic isolates. These strains were resistant to cephalosporins, aminoglycosides, macrolides, nitrofurans, tetracyclines, and sulfonamides. These extensively drug-resistant (XDR) strains were only susceptible to polymyxin (colistin) and quinolone (cephalosporin). This study shows that the virulence traits are shared between plant- and human-isolates of *P. aeruginosa.* More importantly, the occurrence of XDR strains in vegetable crops is a serious global threat.

## P099

# Constructing *vacA* allelic variants of the mouse-colonising *Helicobacter pylori* strain PMSS1

Joanna Stephens[1,2], Darren Letley[1], Joanne Rhead[1], Karen Robinson[1]
[1]University of Nottingham, Nottingham, United Kingdom. [2]Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom

### Abstract

The prevalence of *Helicobacter pylori (Hp)* infection has declined greatly over recent decades, whilst autoimmune and allergic diseases have become more common. Several groups report a protective link between *Hp* infection and multiple sclerosis. Evidence suggests that the VacA toxin plays a role in this, via suppressive effects on dendritic cells. This project aims to identify which polymorphic types of VacA are involved, using *vacA* isogenic mutants in *in vitro* and *in vivo* models.

The *vacA* gene of strain PMSS1 (wild-type (WT) s2/i2 *vacA*) was mutated to create variants with the more active s1/i1 and s1/i2 *vacA* types (PMSS1 s1i1, s1i2). PMSS1 has an active *cag* pathogenicity island (*cag*PAI), so we also constructed *vacA* variants in a *cagE* null background to study toxin effects in the presence and absence of functional *cag*PAI. The mutations were confirmed by PCR and Sanger sequencing, prior to whole genome MiSeq sequencing by Deep Seq. VacA expression levels were compared by immunoblotting, and vacuolating activity was tested on RK13 cells.

The genome sequence of our PMSS1 WT strain was almost identical to the published PMSS1 genome (GenBank CP018823), containing only 4 single nucleotide polymorphisms (SNPs). The mutant strain genomes contained between 14 and 24 unexpected SNPs. VacA was expressed at expected levels, and as expected, the s1i1 mutants had more vacuolating activity than the s2i2 parental strains.

We have constructed *vacA* allelic mutants of PMSS1 and characterised their phenotype *in vitro*. We now aim to study the role of VacA in the interaction between *Hp* and dendritic cells.

## P100

# Using a zebrafish model to examine the importance of the stringent response for *Staphylococcus aureus* pathogenesis

Naznin Choudhury, Rebecca Corrigan
University of Sheffield, Sheffield, United Kingdom

## Abstract

*Staphylococcus aureus* is a human commensal organism with the potential to become opportunistic given the right conditions. During nutrient limitation, bacteria are able to sense, for example, amino acid deprivation and consequently induce the stringent response. The stringent response is a conserved reaction to stress that ultimately promotes bacterial survival, achieved by the production of guanosine penta-/tetraphosphate ((p)ppGpp). (p)ppGpp is a nucleotide signalling molecule responsible for downregulating genes involved in active cellular processes and upregulating genes involved in stress adaptation. (p)ppGpp is synthesised and hydrolysed by RelA/SpoT Homologue (RSH) enzymes. RSH enzymes exist as long mono/bifunctional multidomain proteins or short single domain proteins (short alarmone synthetase/hydrolase (SAS/SAH)). The *S. aureus* genome encodes three (p)ppGpp synthetases: long RSH (Rel) and two SAS (RelP and RelQ). In order to characterise the importance of different synthetase mutants for the survival and virulence of *S. aureus*, we are exploiting a zebrafish infection as well as a human/murine macrophage model. We have constructed single, double and triple synthetase mutant strains and have determined a role for (p)ppGpp in the pathogenesis of *S. aureus.* Future work will focus on elucidating the mechanism behind the requirement of (p)ppGpp for infection.

## P101

# Exploring the faecal bacterial community structure of dogs affected by Cutaneous and Renal Glomerular Vasculopathy (Alabama Rot)

Jack Whitehouse[1], Mark Chambers[1], Joy Leng[1], Roberto La Ragione[1], David Walker[2], Naomi Creelman[2]
[1]University of Surrey, Guildford, United Kingdom. [2]Anderson Moores Veterinary Specialists, Hurley, United Kingdom

## Abstract

Canine cutaneous and renal glomerular vasculopathy (CRGV) is an idiopathic disease characterised by skin lesions and, in some dogs, acute kidney injury. The aetiology of this disease is unknown, hindering the development of specific treatments. In this study, the faecal microbiota from healthy (*n*=96), and CRGV-affected dogs (*n*=104) was investigated using a 16S rRNA community analysis approach. Following DNA extraction, the V4-V5 region of the bacterial 16S rRNA gene was amplified and subsequent amplicons sequenced (MiSeq). To classify sequence taxonomic profiles, sequences were processed using a two-step method generating Operational Taxonomic Units (OTUs) via QIIME2, trained against the Greengenes database (97% similarity level). Data analysis revealed Alpha diversity (measured as observed OTUs) was not significantly different between control and CRGV affected dogs ($p>0.05$). The bacterial community profiles at family level showed CRGV affected dogs had a significantly ($p>0.0025$) higher relative abundance of Enterococcaceae (15.2%), Fusobacteriaceae (12.1%) and Enterobacteriaceae (9.46%), and a lower abundance of Bacteroidaceae (6.01%) and Prevotellaceae (0.18%) compared to healthy control dogs. Linear discriminant analysis on Effect Size (LEfSe) identified bacterial groups of the faecal microbiota that were significantly different between the two groups. LEfSe identified 17 of 107 bacterial groups that were increased in the faeces of dogs affected by CRGV. The groups that were highly associated with CRGV were Leptotrichiaceae and Thermoactinomycetaceae. This study is the first to characterise the faecal microbiota of dogs affected by CRGV.

## P102

# The development of the equine faecal microbiota in the first year of the life of Thoroughbred racehorses

Joy Leng[1], Ruth O'Flaherty[1], Robert Manserg[1], Richard Ellis[2], Falko Steinbach[2,1], Roberto La Ragione[1], Chris Proudman[1]
[1]The University of Surrey, Guildford, United Kingdom. [2]Animal and Plant Health Agency, Addlestone, United Kingdom

## Abstract

Horses rely on the bacteria that reside within their large colon to release short chain fatty acid from their fibrous diet. However, it is not yet clear how early life events including injury and illness could potentially affect the development of the equine gut microbiota. To investigate this, 52 foals, born at five different Thoroughbred studs based in the UK, were enrolled in this study. Nine faecal samples were collected from each foal in the first year of their lives, when they were 2, 8 14, 28, 60, 90, 180, 272 and 365 days old. Bacterial DNA was extracted from all samples and these extracts were submitted for 16S rRNA gene sequencing. The resulting sequencing files were analysed using QIIME2 and R studio (using qiime2r and VEGAN packages). Bacterial diversity (measure as observed OTUs) of the faecal bacterial populations of all foals sampled increased between samples taken at 2 and 90 days old. Bacterial community profiles at order levels showed that, on average foals at 2 days of age had higher levels of *Enterobacteriaceae* and *Fusobacteriales*, whereas samples taken when the foals were 90 days old had higher levels of *Pseudomondales* and *Clostridiales*. The bacterial diversity and bacterial community profiles of samples taken between 90 and 365 days old were not significantly different. This study suggests that by 90 days of age the bacterial community found within the large colon of foals is similar to that of adult horses although there is variation between individual animals.

## P105

# Clostridium clostridioforme; a unique member of the mammalian gut microbiome that directly influences host physiology

Maya Kamat[1], Michael Ormsby[1], Heather Hulme[2], Richard Goodwin[2], Richard Burchmore[1], Leighton Pritchard[3], Daniel Wall[1]
[1]University of Glasgow, Glasgow, United Kingdom. [2]AstraZeneca, Cambridge, United Kingdom.
[3]University of Strathclyde, Glasgow, United Kingdom

## Abstract

Mass Spectrometry Imaging (MSI) revealed novel bacterial metabolites produced by commensal *Clostridium* species in the gut microbiome. These metabolites cross the blood brain barrier and are more abundant in the white matter than elsewhere the brain. The metabolites are structurally similar to carnitine, which transports fatty acids into the mitochondria driving energy production. The metabolites can inhibit mitochondrial function reducing the host cells ability to produce energy.

By employing MSI, metabolite production by the bacteria could be established and concentration and localisation in the brain could be visualised. This allowed further characterisation of the metabolites, which was supplemented with various phenotypic assays. The structure of the metabolites was established as 3-methyl-4-(trimethylammonio)butanoate (3M-4-TMAB) and 4-(trimethylammonio)pentanoate (4-TMAP) via tandem mass spectrometry and correlation spectroscopy NMR.

Whole genome sequencing of *Clostridium clostridioforme* and *Clostridium symbiosum* allowed comparison of the genomes of these metabolite producing strains to those of non-producers. Wider scale analysis of *Clostridium* genomes also allowed potentially novel strains to be identified and provided a baseline for later reclassification work.

Via introduction of the metabolites to neural stem cells from the white matter the potential effect of 3M-4-TMAB and 4-TMAP on the brain could be assessed. Use of proliferation markers Ki67 and Sox2 assessed the ability that these metabolites have to affect stem cell proliferation, a phenotype commonly associated with defects in fatty acid oxidation, which can lead to disease.

Investigation of these unique metabolites will help further elucidate the effect the gut microbiome has on the mammalian health.

## P106

# Bioinformatic exploration of trimethylamine *N*-oxide metabolism in human gut bacteria

Samuel Dawson, Lesley Hoyles
Nottingham Trent University, Nottingham, United Kingdom

## Abstract

Trimethylamine *N*-oxide (TMAO) is a microbial metabolite that has been shown to have protective effects on the blood–brain barrier, while elevated serum levels of TMAO and its precursors have been linked to cardiometabolic diseases in Western populations. Previous work examined the prevalence of TorA to determine which groups of bacteria were responsible for the metabolism of TMAO in the human gut. This study examined 6 TMAO metabolism pathways to provide a more in-depth analysis of bacterial TMAO metabolism.

Using NCBI BLAST+ 31,410 publically available genomes and 4644 human gut reference genomes (HGRG) were screened for the presence of 20 proteins that were found to be relevant to bacterial TMAO metabolism. These results were then filtered for hits with >90% coverage and >70% identity.

Results showed that Tor proteins were largely limited to members of the *Enterobacteriaceae*, mostly appearing in *Escherichia coli* and *Citrobacter* spp. >1% of 9898 *Klebsiella* spp. genomes examined encode any Tor proteins, despite previous work highlighting *Klebsiella* spp. as one of the prevalent genera encoding TorA. Dms proteins were much more prevalent than TorA in other genera of bacteria, along with MsrP and BisC. 118 of the HGRGs were found to encode for at least 1 TMAO metabolism protein.

Overall, this work highlights the need for more comprehensive methods to be used to examine large genomic and metagenomic datasets and the need for *in vitro* work to be done alongside *in silico* analyses to improve functional annotations and our understanding of the roles of gut bacteria.

## P107

# Evaluating the antimicrobial potential of putative bacterial probiotic strains isolated from the athlete gut.

Laura Wosinska[1,2,3], Paul D. Cotter[2,3], Orla O`Sullivan[2,3], Caitriona M. Guinane[1]
[1]Munster Technological University, Cork, Ireland. [2]Teagasc Food Research Center, Moorepark, Fermoy, Ireland. [3]APC Microbiome Ireland, Cork, Ireland

### Abstract

In recent years exercise has been shown to confer many health benefits on the human body. Studies have shown that physical activity can positively impact our cardiovascular health as well as alleviate fatigue and inflammation. It has also been demonstrated that physical fitness can influence the gut microbiome and, as a result, health. Physical activity has been shown to alter the gut microbial community by increasing and/or decreasing the abundance of certain bacterial taxa. These alterations in microbial community composition can be exploited to assess potential probiotic traits with applications in athlete cohorts and the general population.

In this study, 11,000 colonies were isolated from an existing biobank of athlete faecal samples using a variety of rich and selective media and culture conditions. Cultured isolates were assayed for probiotic traits including bacteriocin/antimicrobial peptide production. Isolated colonies were overlaid with growth medium seeded with indicator strains (*Lactobacillus bulgaricus, Listeria innocua, Clostridium difficile*). This yielded 342 isolates that exhibited putative antimicrobial activity in laboratory media. These were further investigated using well diffusion and spot-based antimicrobial assays. The most potent strains were taken forward for MALDI-TOF mass spectrophotometric analysis of the antimicrobial compounds and whole genome sequencing to assist with the further profiling of probiotic traits.

The results highlight the potential of the athlete microbiome as a source of novel probiotic strains that could be employed to control infection in the athlete gut, but also a potential to be exploited in other ways and to be applied to a wider non-athlete population.

## P108

# Metagenomic Analysis of Functional Bile Acid Genes and Bile Acid Composition in Faecal Samples of Bile Acid Diarrhoea Sufferers

Evette Hillman[1,2], Danielle Carson[1], Lijiang Song[2], Martin Fritzsche[1], Ryan Mate[1], Sjoerd Rijpkema[1], Elizabeth Wellington[2], Ramesh Arasaradnam[2,3], Gregory Amos[1]
[1]National Institute for Biological Standards and Control, South Mimms, United Kingdom. [2]The University of Warwick, Coventry, United Kingdom. [3]University Hospital Coventry & Warwickshire, Coventry, United Kingdom

## Abstract

Bile Acid Diarrhoea (BAD) is a widespread gastrointestinal disease, estimated to affect 1% of the population in the United Kingdom alone. BAD is associated with excessive bile acid synthesis secondary to a gastrointestinal or idiopathic disorder, therefore, decreasing bile synthesis can lead to reduced symptoms. Bacteria are essential in transforming primary bile acids into secondary bile acids. The profile of an individual's bile acid pool is central in maintaining bile acid homeostasis as bile acids regulate their own expression. Indeed, examining the gut microbiome to determine the bile acid transforming potential of the microbiota could help elucidate key bacterial strains potentially responsible for causing BAD.

BAD is often misdiagnosed as irritable bowel syndrome (IBS), with up to a third of IBS sufferers having BAD. Human stool samples from 26 BAD patients, ~ 80 IBS patients and ~100 healthy donors (controls) were provided by collaborators at the University Hospitals Coventry & Warwickshire (Prof. Arasaradnam). Firstly, the microbiome was analysed following DNA extraction and shotgun sequencing at a depth of 50 million reads per sample. Metagenomic data was used to analyse both the taxonomic structure of the microbiome, with targeted analysis of BSH gene and BAI genes used to determine the microbial potential for secondary bile acid synthesis. Secondly, the bile acid composition for each sample was determined using UPLC-MS.

Here, we will report the results from the analysis of the human faecal microbiome and bile acid profiles and explore the role of the microbiome in bile acid homeostasis in IBS and BAD.

## P109

# *In Silico* Genomic and Metabolic Atlas of *Lactobacillus reuteri* DSM 20016: An Insight Into Human Health

Paisleigh Smythe, Georgios Efthimiou
University of Hull, Hull, United Kingdom

## Abstract

*Lactobacillus reuteri* is a facultative anaerobic bacterium that is naturally found within various body areas, including the small intestine, oral cavity and vagina. It is a well-established probiotic, already on the market, and is known to produce several beneficial compounds, such as vitamins, enzymes and amino acids, as well as anti-inflammatory and antimicrobial products. However, the biosynthetic pathways and the microbial factors that are behind these bioprocesses are currently poorly characterised and understood.

This study employed a variety of genomic and proteomic tools and databases (UniProt, KEGG, STRING, BLAST) to search, identify and interpret the genome and proteome of *L. reuteri* DSM 20016, specifically aiming to shed light on key mechanisms that lead to the biosynthesis of microbial compounds that are beneficial for human health.

194 key genes were identified and localised on the *L. reuteri* genome. They are involved in the biosynthesis of short chain fatty acids (SCFA) that are known for their anti-inflammatory effects, the metabolism of amino acids and vitamins, the production of histamine and the formation of an antimicrobial and antioxidant product known as reuterin. Adherence factors by to which *L. reuteri* DSM 20016 attaches to gut epithelial cells were also identified. This is important, as better colonisation usually leads to longer health benefits by probiotics.

Characterisation of the metabolic abilities of this strain could help in the development of new probiotic-based treatments against gut inflammation, certain cancers, diarrhoea and acute colitis.

## P110

# An *in silico* genomic and proteomic study of *Bifidobacterium longum* subsp. *infantis* ATCC 15697 with a focus on its health benefits

Mutale Kangote, Georgios Efthimiou
University of Hull, Hull, United Kingdom

### Abstract

The human gut hosts a prevalent population of *Bifidobacterium* spp., a genus of Gram-positive anaerobic bacteria known for their significant health benefits. Bifidobacteria are present in many probiotic supplements currently on the market, therefore they also have a direct economic importance for the blooming probiotics industry. The aim of this *in silico* project was to identify genes and proteins that help *Bifidobacterium infantis* ATCC 15679 to produce beneficial metabolites and effectively colonise the gut, hence promoting human health.

Various bioinformatic tools were used, such as STRING for analysing the interactions between key proteins, BLASTp for comparing similarities in the amino acid sequences and UNIPROT for confirming protein sequences and functions. In addition, databases such as KEGG and NCBI were employed for retrieving genomic and literature information.

Thirty genes related to human health were identified. STRING analysis showed interesting interactions between 12 out of the 30 proteins encoded by these genes. The most important key genes were: Blon_1731 (biosynthesis of short chain fatty acids that prevent gut inflammation), Blon_2334 (lactase), Blon_0388 (riboflavin and folate synthesis), Blon_0136 (pantothenic acid production), and finally Blon_1004 and Blon_0855 (biofilm formation). The last two factors lead to better gut colonisation and subsequently to improved long-term health effects.

This study led to the construction of a very useful genomic and functional atlas for *B. infantis* ATCC 15679 that will help developing new strategies for improving the beneficial properties and the colonisation efficiency of this strain by metabolic engineering approaches.

## P111

# Bacterial and Protozoan Lipoxygenases: an Evolutionary Link to Multicellularity and Virulence

Georgy Kurakin[1], Anna Samoukina[1], Nadezhda Potapova[2]
[1]Tver State Medical University, Tver, Russian Federation. [2]Institute for Information Transmission Problems of the Russian Academy of Sciences (Kharkevich Institute), Moscow, Russian Federation

## Abstract

Lipoxygenases are enzymes converting polyunsaturated fatty acids into their hydroperoxides. In animals, plants, algae and fungi, these hydroperoxides are further converted into oxylipins that serve as cell-to-cell signaling compounds. Lipoxygenases and lipoxygenase-derived oxylipins have been described also in a small percentage of bacteria and protozoa, but their functions are largely unknown. Experimental data on microbial lipoxygenase functions are scarce.

We performed a bioinformatics study to find associations between lipoxygenase presence in microbes and their ecophysiological traits. Thus, we tried to infer possible functions of bacterial and protozoan lipoxygenases and their evolution. We used simple statistical analysis and phylogenetic analysis by the means of phylogenetic trees and networks. We found that lipoxygenase presence is statictically and evolutionary associated with microbial taxa forming primitive multicellular structures, like cyanobacteria, myxobacteria, slime molds and oomycetes. Our evolutionary study has shown that lipoxygenase spreaded between multicellular and multicellular-like taxa by horizontal gene transfer, and the consecution of these transfers correlate well with origins of multicellularity. We concluded that lipoxygenases originated in bacteria to provide cell-to-cell signaling and was borrowed by eukaryotes for the same role.

We have also identified an association of lipoxygenases with virulence in dangerous nosocomial and opportunistic pathogens. Our results show that lipoxygenases may be an emerging virulence factor.

This research is published in the journal *Biochemistry (Moscow)*:

Kurakin G.F., Samoukina A.M., Potapova N.A. (2020) Bacterial and Protozoan Lipoxygenases Could be Involved in Cell-to-Cell Signaling and Immune Response Suppression. *Biochemistry (Mosc)*; 85(9): 1048-1071. doi: 10.1134/S0006297920090059. PMID: 33050851.

## P112

# The genome methylation landscape of ancyromonads, a deep-branching eukaryotic lineage

Jazmin Blaz[1], Luis Javier Galindo[1], Guifré Torruella[1], Naoji Yubuki[1], Maria Ciobanu[1], Aaron Heiss[2], John Burns[2], Eunsoo Kim[2], Purificación López-García[1], David Moreira[1], Laura Eme[1]
[1]Unité Ecologie Systématique et Evolution, CNRS, Université Paris-Saclay, Orsay, France. [2]American Museum of Natural History, 200 Central Park West, New York, USA

## Abstract

Genomes contain information superimposed to the DNA sequence. This information, the epigenome, impacts the local activity, stability and evolution of the genome and underlies a great extent of the complexity and diversity displayed by eukaryotes. One of the most ancient and phylogenetically widespread components of the epigenome consists of the DNA methylation marks. In eukaryotes, the patterns of DNA methylation and the pathways responsible for them have diversified across the different phylogenetic supergroups. However, this diversity and its biological role remain unexplored in most of the lineages of the tree of eukaryotic life.

To gain insight into the macroevolution of the methylation landscape across eukaryotes, we have sequenced the first genomes and methylomes of diverse representatives of the deep-branching clade Ancyromonadida. Combining long and short-read sequencing technologies, we generated the draft genomes of Ancyromonas sigmoides (str. B70) and Fabomonas mesopelagica (str. A153). Furthermore, using bisulfite sequencing we characterized the overall level and distribution of 5-methylcytosine marks across these genomes. Our preliminary results point to a difference between the total interspersed repeat content of these two species (19.6% for B70 and 0.68% for A153) but similar global levels of methylation (1.4 and 1.93% of methylated cytosines respectively). In combination with transcriptome data, we will analyze the relationship between the patterns of DNA methylation and genomic organization and expression in these species. Our data represents the first exploration of the epigenome in this obscure branch of eukaryotes and provides an opportunity to investigate the deep evolution of this hallmark of eukaryotic genome regulation.

## P113

# The evolution of mechanisms for dividing labour in non-clonal populations

Ming Liu[1], Guy Cooper[1,2], Stuart West[1]
[1]Department of Zoology, University of Oxford, Oxford, United Kingdom. [2]St. John's College, Oxford, United Kingdom

## Abstract

Division of labour, where cooperating individuals go through specialisation to perform complementary tasks, can be found across the tree of life and achieved through different mechanisms. These mechanisms include random and coordinated specialisation, and it is not clear why organisms use different mechanisms to divide labour. Previous research has focused on clonal groups, however, as many non-clonal microbial species show division of labour, there is no theoretical prediction for these systems. Here, we developed both analytical and simulative models to examine the evolution of dividing mechanism in non-clonal population. Both models show (1) division of labour is less common, (2) proportion of helpers drops, and (3) random specialisation becomes more dominant as relatedness decreases. These results arise from more potential conflicts between group members. Although additional empirical data is needed to verify our predictions, we provide the first theoretical framework for the evolution of mechanisms under non-clonal life history settings.

**P117**

# Investigating molecular mechanisms for phosphorus signalling in the marine diatom, *Phaeodactylum tricornutum*

Yasmin Meeda[1,2], Dr Adam Monier[2], Dr Glen Wheeler[1], Dr Katherine Helliwell[1,2]
[1]The Marine Biological Association, Plymouth, United Kingdom. [2]The University of Exeter, Exeter, United Kingdom

## Abstract

Marine diatoms are ubiquitous microalgae that contribute around one-fifth of global $CO_2$ fixation. Phosphorus, an essential element needed for all living organisms, is often found in scarce supply in many marine ecosystems and can thus limit diatom growth and productivity. As diatoms compete with other phytoplankton, they have evolved metabolic mechanisms to cope with prolonged periods of phosphorus limitation. Diatoms are also able to rapidly sense when the phosphorus supply increases and regulate their metabolism accordingly. This suggests that diatoms have evolved sophisticated mechanisms for sensing environmental phosphorus availability. Recent evidence has identified that diatoms can sense phosphorus using a $Ca^{2+}$-dependent signalling pathway, however, the molecular machinery underpinning this pathway remains unknown.

Here, we investigated the role of $Ca^{2+}$-dependent kinases (CDPKs) in governing recovery responses of phosphorus-starved diatom cells to phosphorus resupply. Employing CRISPR-Cas9 gene-editing approaches, we generated gene knockout lines of *PtCDPK1* in the marine diatom, *Phaeodactylum tricornutum*. We are now employing a range of physiological approaches to monitor the capacity of these mutants to grow in different phosphorus regimes. Additionally, we have explored the broader distribution of CDPKs across diatom taxa. This work has demonstrated that CDPKs are found widely distributed across the diatom lineages. Moreover, this family of proteins has expanded in certain diatom species, suggesting that they may have evolved diverse functional roles. This work aims to provide new insight into how this important group of marine algae are able to thrive in regions of pulsed nutrient supply.

## P118

# Unravelling the function of the unusual antioxidant ergothioneine in photosynthetic organisms

Isobel Cole[1,2], Glen Wheeler[2], Katherine Helliwell[1,2], Debbie Salmon[1], Nicholas Smirnoff[1]
[1]University of Exeter, Exeter, United Kingdom. [2]Marine Biological Association, Plymouth, United Kingdom

## Abstract

Photosynthetic eukaryotes produce a range of antioxidants to protect them from reactive oxygen species produced during photosynthesis. It has recently been discovered that some plants and algae accumulate ergothioneine, a thione/thiol compound derived from the amino acid histidine, but its role in these organisms is not currently understood. As a potential antioxidant, it could be important in the protection against environmental stresses. Ergothioneine is also effective at binding potentially toxic metals such as copper, nickel and cobalt, suggesting additional cytoprotective roles. However, there is currently little known about the occurrence, synthesis and physiological functions of ergothioneine in photosynthetic organisms. We will examine a diverse range of algal and plant lineages to determine their ability to accumulate ergothioneine. A combination of phylogenetic analysis, gene knockouts using CRISPR-Cas/amiRNA and expression of candidate genes will then be used to investigate the biosynthetic pathways in selected species and characterise the enzymes involved in ergothioneine synthesis. Biochemical and physiological characterisation of wild type and ergothioneine deficient mutants will be used to investigate the protective role of ergothioneine.

**P119**

# Multi-omics analysis of the specialised metabolism of two novel *Pseudonocardia* spp. isolated from the deep Southern Ocean

Jonathan Parra, Katherine R. Duncan
Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom

## Abstract

Multidrug-resistant pathogens have become a global threat. In this context, filamentous Actinobacteria has been proven to be an exceptional source of antimicrobial metabolites. In particular, rare actinomycetes isolated from marine environments have been proposed as a potential source of yet untapped specialised metabolites. In this study, two novel species, *Pseudonocardia abyssalis* sp. nov. and *Pseudonocardia oceani* sp. nov, isolated from deep Southern Ocean sediments are described, both in terms of their phenotypic and genomic characterization. Furthermore, the genomic architecture, with a focus on Biosynthetic Gene Cluster (BGC), across eight strains belonging to the two novel species were investigated. A total of 14 Gene Cluster Families (GCF) were identified, of which five GCFs comprise BGCs from both species, and nine were specific to each species. Moreover, a correlation of GCFs to phylogeny was observed. Following genome analysis, a comparative mass-spectrometry based metabolomics analysis was carried out with one strain from each new species, as well as *Pseudonocardia ammonioxydans* and *Pseudonocardia sediminis*, also of marine origin. The metabolomics profiles agreed with the GCF distribution, where a group of ubiquitous metabolites were produced by both new *Pseudonocardia* spp., while groups of species-specific metabolites were also detected. This metabolic-repertoire was found to be elicitated through the addition of *N*-acetyl glucosamine (GlcNAc), revealing chemically-inducible bioactivity against the fungi *Candida albicas* and multidrug-resistant *Candida auris*. These results showcase the power of a combined genomic-metabolomics approach to investigate rare-actinomycetes from understudied locations and have uncovered a wealth of both biosynthetic and chemical diversity for further investigation.

## P120

# Hydrocarbon-degrading microorganisms associated with photobioreactors-grown microalgae *Pavlova lutheri* and *Nannochloropsis oculata*

Tatyana N. Chernikova[1,2], Rafael Bargiela[1], Stepan V. Toshchakov[3], Vignesh Shivaraman[1], Evgenii A. Lunev[4], Michail M. Yakimov[5], David N. Thomas[6], Peter N. Golyshin[1,2]
[1]School of Natural Sciences, Bangor University, Bangor, United Kingdom. [2]CEB-Centre for Environmental Biotechnology, Bangor University, Bangor, United Kingdom. [3]National Research Centre "Kurchatov Institute", Moscow, Russian Federation. [4]Immanuel Kant Baltic Federal University, Kaliningrad, Russian Federation. [5]Institute for Biological Resources and Marine Biotechnology, IRBIM-CNR, Messina, Italy. [6]School of Ocean Sciences,Bangor University, Bangor, United Kingdom

## Abstract

Marine photosynthetic microalgae produce oleophilic compounds, such as alkanes, long-chain fatty acids, and alcohols and were reported to host naturally occurring hydrocarbon-degrading specialist bacteria. However, little is known about the taxa of microalgae that can host such bacteria, composition of microalgae-associated microbial consortia and the responses of these consortia to the petroleum. This study assessed structures of microbial communities, particularly those with hydrocarbon degradation abilities for both aliphatic and aromatic hydrocarbons associated with two photobioreactors-grown cultures of ubiquitous microalgae, *Pavlova lutheri* and *Nannochloropsis oculata,* using culture-independent (barcoded SSU rRNA amplicon sequencing Illumina MiSeq and Oxford MinION platforms) and cultivation approaches. While bioreactor communities contained predominantly *Proteobacteria* (class *Alphaproteobacteria*), *Bacteroidetes* and *Planctomycetes*, the addition of crude oil resulted in stark changes in both microalgal cultures. More than 25% of the total reads in crude oil enrichments were derived from members of genera *Alcanivorax* and *Marinobacter,* the renowned hydrocarbonoclastic bacteria, which were detected in less than 0.5 % reads in bioreactor communities. Using cultivation, 48 bacterial non-redundant strains were isolated and identified to belong to the genera *Alcanivorax, Marinobacter*, *Thalassospira, Hyphomonas, Halomonas, Marinovum, Roseovarius, Oleibacter*. The results of this study showed that microalgae *Pavlova lutheri* and *Nannochloropsis oculata* represent a natural reservoir of diverse taxa of potent hydrocarbon-degrading bacteria.

## P121

# Examining Phosphate Physiology in Coccolithophore Life Cycle Stages

Daniela Sturm[1,2], Glen Wheeler[1], Colin Brownlee[1], Toby Tyrell[2]
[1]Marine Biological Association, Plymouth, United Kingdom. [2]University of Southampton, Southampton, United Kingdom

## Abstract

Despite the significance of coccolithophores for biogeochemical cycling, much of their ecology remains poorly understood. In particular, their elusive haplo-diplontic life cycle has been given little attention in the literature. Yet, it significantly impacts the extent of coccolithophore calcification, their vertical and horizontal distribution in the ocean, and potentially their global success.

The aim of this project is to establish physiological differences between the life cycle stages of the ecologically important coccolithophores *Coccolithus braarudii* and *Calcidiscus leptoporus* in response to changes in the essential macronutrient phosphorus (P). Haploid coccolithophores are more commonly found in P-limited environments, raising the question which underlying mechanisms cause this variable distribution between life stages.

An initial investigation of growth, photophysiology, calcification, and storage of polyphosphate under P-limited conditions will determine which parts of coccolithophore physiology are heavily impacted by P-limitation. An *in silico* analysis of P acquisition and transport genes in coccolithophores and other closely related eukaryotes will highlight differences in P physiology among these groups. Comparing proteins expressed in haploid and diploid coccolithophores under P-limitation will then be used to reveal whether P acquisition strategies differ between the two life stages.

These results will further shed light on potential adaptations of haploid and diploid coccolithophores to different ecological niches. A profound understanding of coccolithophore physiology is vital to revealing their evolutionary success and their impact on ocean biogeochemistry and ecology.

## P122

# Microbial mediators of the marine nitrogen cycle: the role of trace metals in the niche separation of ammonia-oxidising archaea and bacteria

Roxana Shafiee, Poppy Diver, Joseph Snow, Qiong Zhang, Rosalind Rickaby
University of Oxford, Oxford, United Kingdom

## Abstract

Ammonia oxidation by archaea and bacteria (AOA and AOB, respectively) is the first step in nitrification, which produces the nutrient nitrate ($NO_3^-$) in the oceans. Marine ammonia oxidation is dominated by AOA, in part due to their greater affinity for ammonium compared with AOB. Yet the strategies that allow AOB to persist in the nutrient-poor oceans despite being poor competitors for ammonium remain enigmatic. Trace metal micronutrients shape the competition and niche separation of marine primary producers, but their role in the niche differentiation of marine AOA and AOB has yet to be examined. In this study, we compared the physiological response of AOA and AOB isolates, *Nitrosopumilus maritimus* and *Nitrosococcus oceani,* to the availability of the bio-essential metals, copper and iron. We found that *N. oceani* has a greater affinity for unchelated iron compared with *N. maritimus* which we posit may allow AOB to inhabit shallower, euphotic waters where ammonium supply is high, but competition for iron is rife. In contrast to *N. oceani*, *N. maritimus* has a greater affinity and toxicity threshold for unchelated copper, providing a potential explanation to the greater success of AOA in the marine environment where copper availability can be highly variable. Using comparative genomics, we predict that the proteomic and metal-transport basis giving rise to contrasting physiologies in isolates is widespread across phylogenetically diverse marine AOA and AOB which are not yet available in pure culture suggesting our work on marine isolates is representative of *in situ* AOA and AOB communities.

## P123

# Genomics to Investigate Risks of Toxin-Producing Vibrio in Irish Shellfish

Katie Boyle[1], Alison Levy[1], Aoife Boyd[1], Fiona Walsh[2]
[1]National University of Ireland Galway, Galway, Ireland. [2]Maynooth University, Maynooth, Ireland

## Abstract

Intro: *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* are marine pathogens that cause gastroenteritis in humans after the consumption of infected shellfish. This project investigates the occurrence and distribution of pathogenic and toxigenic Vibrio in farmed Irish shellfish and the association with seasonal fluctuations in temperature and salinity to determine the potential risk to shellfish production and human health .

Methods: We developed a programme of monthly shellfish harvesting and environmental monitoring at shellfish farming locations on the West coast of Ireland. Shellfish, water and sediment samples were analysed for Vibrio using culture and genetic approaches. Vibrio isolates possessing toxigenic virulence genes associated with disease in humans were identified via PCR. Furthermore, as Vibrio has recently been found to produce tetrodotoxin (TTX), the presence of TTX-producing Vibrio will be determined through immunological assay.

Results: Our Vibrio monitoring programme started in November 2020. So far, we have detected *V. parahaemolyticus* at shellfish farm locations, in sediment and oysters. Neither *V. cholerae* nor *V. vulnificus* were detected. No pathogenic Vibrio were identified in mussels. PCR analysis identified the presence of Type Three Secretion System toxigenic genes in 8 of 10 *V. parahaemolyticus* isolates. The effect of temperature on the presence of *V. parahaemolyticus* was also apparent as it was undetected in waters less than 10°C.

Conclusion: By identifying the risks posed by pathogenic and toxin producing Vibrio, measures will be proposed and put in place to minimise and overcome these threats to both the shellfish consumers and shellfish producers.

## P124

# Synergistic biodegradation of a compostable film by a marine microbial community.

Ingrid E. Meyer-Cifuentes, Başak Öztürk
The Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany

## Abstract

### Background

The need for replacing conventional plastics has led to an increase of the use biodegradable plastics. Most biodegradable plastic materials are certified for compostability, and their degradation mechanisms by marine bacterial communities, are still largely unknown.

### Methods

Bacterial communities that degrade a poly (butylene adipate-co-terephthalate)-based biodegradable film (PF) were enriched from marine samples. DNA, RNA and proteins were extracted simultaneously from the biofilm and free-living bacteria. Genes of hydrolases similar to the ones involved in polyethylene terephthalate (PET) and monoester mono-2-hydroxyethyl terephthalate (MHET) degradation (PETase and MHETases, respectively) were detected. A MHETase-like gene (Mle046) was then recombinantly expressed. The activity of Mle046 was tested against the end product of PET and PF degradation: MHET and 4-(4-hydroxybutoxycarbonyl) benzoic acid (Bte), respectively. The optimal incubation temperature and pH of Mle046 activity was determined.

### Results

PETase-like (Ples) and MHETase-like (Mles) hydrolases and other enzymes needed for PF degradation were expressed within the microbial community. Within the biofilm, Ples were abundant and upregulated while Mles and terephthalate dioxygenases were abundant in the free-living fraction. Mle046 was the only Mle produced in this fraction and it was highly expressed. The purified Mle046 could degrade MHET and Bte. The optimum temperature of Mle046 activity was 20°C.

### Conclusion

PF degradation is achieved synergistically by labour division among film-attached and free-living bacteria. Understanding the biodegradability of these plastics will facilitate the development of more degradable materials. In addition, the discovery of new PETases- and MHETases-like enzymes will enable their future use in plastic recycling.

## P125

# Diversity and dynamics of particle-associated and free-living bacteria in eelgrass (*Zostera marina*) bed along the coast of Japan

Md Mehedi Iqbal[1], Masahiko Nishimura[1], Masayoshi Sano[1,2], Susumu Yoshizawa[1]
[1]The University of Tokyo, Tokyo, Japan. [2]National Institute of Polar Research, Tokyo, Japan

## Abstract

As the seagrass leaves remain underwater, the primary source of leaf microbes is considered to be seawater heterotrophic bacterioplankton, which possess the ability to degrade biopolymers and are known to attach to surface and form biofilms. In this study, 16S rRNA gene amplicon sequencing was used to assess bacterial diversity and dynamics of the particle associated (PA) and free-living (FL) fraction of the seagrass-covering seawater (inside) and bulk seawater (outside) among different seagrass bed around Japan. Samples were collected from the three *Zostera marina* beds (Ikuno-shima Is., Hiroshima; Nanao Bay, Ishikawa; Mutsu Bay, Aomori Prefecture) around Japan during summer (June-August 2015; July 2016). Prokaryotic DNA was extracted from samples using a FastDNA spin kit according to the manufacturer's protocol. After extracting DNA, 16S ribosomal RNA (16S rRNA) genes were sequenced by Illumina Miseq platform. The Results showed that PA bacterial communities had a higher ($p < 0.001$) diversity than FL ones. Compared to the outside of the seagrass bed, the inside had lower diversity both in PA and FL fraction. Taxonomic analysis revealed a different community composition between lifestyle (PA vs FL) and sampling point (inside vs outside). Differential abundance analysis showed that PA were significantly enriched in a diversity of Cyanobacteria (*Synechococcaceae), Saprospiraceae and Hyphomonadaceae*. Conversely, FL were more abundant in Gammaproteobacteria (including *Halomonadaceae*, *Alteromonadaceae), Microbacteriaceae, Campylobacteraceae, Pelagibacteraceae,* Acidimicrobiia *(OCS155*). The present data provide a comprehensive description of the PA and FL microbial community in the seagrass bed and can be useful for better understanding the seagrass microbe interactions.

## P126

# Characterising the diversity, abundance, and nature of diatom-bacteria interactions in the Western English Channel

Laura Branscombe[1,2], Michael Cunliffe[1,2], Willie Wilson[1,2], Katherine Helliwell[1,3]
[1]Marine Biological Association of the UK, Plymouth, United Kingdom. [2]University of Plymouth, Plymouth, United Kingdom. [3]University of Exeter, Exeter, United Kingdom

## Abstract

Diatoms are a major group of photosynthetic microalgae, which often dominate marine phytoplankton communities. Due to their ubiquity and abundance, diatoms are widely recognised to have global-scale impacts on marine biogeochemical cycles. Furthermore, certain diatom species can form large, toxic blooms that can heavily influence phytoplankton ecology.

The co-existence of diatoms with major planktonic groups such as marine bacteria has resulted in a spectrum of complex interactions, from synergistic to antagonistic. The importance of biotic interactions in shaping diatom growth, physiology and bloom regulation is becoming increasingly recognised. Some studies indicate that diatoms may respond to the chemical signatures of their microbial neighbours by altering their physiology or metabolism, leading to responses ranging from increased toxicity, to algal cell lysis and subsequent population decline. Despite the ecological significance of such interactions, their diversity, abundance, and role in governing diatom cell biology are not fully understood.

In this project, we aim to characterise interactions between ecologically relevant diatoms and antagonistic bacteria. By isolating algicidal bacteria from the Western English Channel, we are examining the diversity and abundance of diatom algicidal bacteria in this highly productive ecosystem. In addition, we are characterising the physiological impacts of algicidal bacteria on a range of diatoms. Furthermore, by employing a novel molecular toolkit we have developed for model diatom *Phaeodactylum tricornutum*, we will investigate the early intracellular signalling responses of diatoms to associated and algicidal bacteria, allowing us to gain a deeper understanding of diatom adaptations to algicidal bacteria and how they are regulated.

**P127**

# Prevalence of antimicrobial resistance in bacteria from wild and captive pinnipeds: a systematic review and meta-analysis.

Lauren Arkoosh, Anne Savage, Kimberley Bennett, Scott Cameron
Abertay University, Dundee, United Kingdom

## Abstract

Antimicrobial Resistance (AMR) is a pressing concern for human and animal health. Bacteria containing resistance are ubiquitous in the environment, and can spread rapidly because of human and industrial waste, agriculture, and aquaculture. Many of the waste products from these processes eventually make their way to ocean waters, providing AMR-containing bacteria opportunity to disseminate. Pinnipeds are apex predators in UK waters, sampling the entirety of the water column when they forage, making them a useful sentinel species to examine AMR presence in marine environments. Here we extracted data from studies on all pinnipeds in all wild, captive or stranded contexts, including any sample type collected (e.g. faecal, nasal swab, gut content) and assessed for AMR. Fixed search terms were used to identify and extract prevalence data from studies examining AMR in pinnipeds. We examine what AMR has been tested and in which bacterial species; their prevalence, including the extent of multiple resistances; the difference in prevalence and the type of AMR among pinniped species, sample types, regions, or settings and whether there has been change over time. Finally, we assess methodological bias toward aerobic or pathogenic bacteria investigated and whether there is bias in the health status of sampled animals or in the locations investigated. This information provides an assessment of the extent of past and current information and a prevalence estimate for AMR in these marine sentinel species for use by scientists and policy-makers to inform future work and assessments regarding AMR in the marine environment.

## P128

# Iron, zinc and cobalt limitation on cyanobacterial alkaline phosphatases in the subtropical North Atlantic

K. Kunde[1], N. A. Held[2], C. E. Davis[3], N. J. Wyatt[1], E. M. S. Woodward[4], M. McIlvin[5], C. M. Moore[1], B. S. Twining[6], M. A. Saito[5], A. Tagliabue[3], C. Mahaffey[3], M. C. Lohan[1]
[1]University of Southampton, Southampton, United Kingdom. [2]Swiss Federal Institute of Technology, Zurich, Switzerland. [3]University of Liverpool, Liverpool, United Kingdom. [4]Plymouth Marine Laboratory, Plymouth, United Kingdom. [5]Woods Hole Oceanographic Institution, Woods Hole, USA. [6]Bigelow Laboratory for Ocean Sciences, East Boothbay, USA

## Abstract

Alkaline phosphatases provide an important pathway for phytoplankton to access the vital nutrient phosphorus from the dissolved organic phosphorus (DOP) pool, when the more bioavailable dissolved inorganic phosphorus (DIP) is depleted, such as in the surface subtropical North Atlantic. However, the requirement for zinc (Zn), cobalt (Co) or iron (Fe) co-factors in these enzymes gives rise to potential metal-phosphorus co-limitation of primary production. Applying calibrated quantitative proteomic measurements of alkaline phosphatases of two key primary producers, *Synechococcus* and *Prochlorococcus*, in metal-amended bioassays *in-situ*, we demonstrate the localised metal limitation on alkaline phosphatases at two contrasting biogeochemical sites within the subtropical gyre. In the western basin, the additions of Zn or Co stimulated the production of a *Synechococcus* PhoA-type alkaline phosphatase, while in the eastern basin the addition of Fe stimulated the production of a *Prochlorococcus* PhoX-type alkaline phosphatase. As field-based evidence of Co limitation on the *Synechococcus* PhoA, our results suggest the effective substitution between Zn and Co in this protein. Using cellular metal stoichiometry, we show that *Synechococcus* PhoA could be a major sink for cellular Co but not for Zn, and hence that it could be sensitive to ambient Co availability globally. The predicted exacerbation of phosphorus stress and altered trace metal availability in the future ocean are currently met with a limited baseline understanding of marine alkaline phosphatase dynamics. Our results highlight the timeliness of combining trace metal chemistry, biological rate measurements, and cell quotas with proteomics on the basin scale.

**P129**

# Bacterial diversity of two types of Wagashi, a traditional Beninese cheese, using High-Throughput Amplicon Sequencing

Gwladys S. KOMAGBE[1,2], Philippe Sessou[2], Souaïbou Farougou[2], Jacques Mahillon[1]
[1]Laboratory of Food and Environmental Microbiology, Earth and Life Institute, Université Catholique de Louvain, Louvain-la-Neuve, Belgium, Louvain-la-Neuve, Belgium. [2]Research Unit on Communicable Diseases, Laboratory of Research in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Benin, Abomey-Calavi, Benin

## Abstract

*Wagashi*, also called *Gassiré* in the local Fulfulde language, is a traditional cheese produced from cow milk in Benin. For its preparation, milk is heated and coagulated with *Calotropis procera* extract. After coagulation, the heated curd obtained is drained, moulded and stained, or not, using *Sorghum vulgare* or *Sorghum codatum* panicles. It is the most commonly consumed dairy product as surrogate for meat or fish. In order to get better insights into the bacterial diversity of *Wagashi*, Illumina MiSeq amplicon sequencing targeting the V1-V2 region of the bacterial 16S rRNA gene was performed on two different *Wagashi* types (stained and not stained) from Benin. The results showed that the *Lactobacillaceae* (60%) and *Streptococcaceae* (38%), were the most abundant bacteria in the unstained cheese, whereas *Streptococcaceae* (89%) and *Bacillaceae* (10%) were the most prevalent in stained cheese. Moreover, at the species level, the microbial community structures of stained and unstained cheeses were significantly different. The main differentiating species were *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus fermentum* in unstained cheese, compared to *Streptococcus infantarius*, *Aeromonas* sp., *Bacillus cereus*, *Bacillus subtilis* and *S. thermophilus* in stained cheese. This work provides valuable insights into microbiology of cheese *Wagashi* and shows that the staining of this dairy product affects its bacterial composition. In particular, the presence of potential useful Lactobacilli and Streptococci in unstained cheese is to be further investigated for selecting functional bacteria for potential biotechnology applications. On the contrary, the presence of Bacilli in stained cheese needs additional study to detect potential foodborne strains or pathotypes.

## P130

# Genetic Tools for the Thermophilic Acetogen *Moorella thermoacetica*

Barbara Bourgade[1,2], James Millard[2], Nigel P. Minton[2], M. Ahsanul Islam[1]
[1]Loughborough University, Loughborough, United Kingdom. [2]BBSRC/EPSRC Synthetic Biology Research Centre, Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom

## Abstract

The environmental impacts of petrochemical industries and the finite nature of fossil fuels has led to an urgent need to find alternative routes to fuel and chemical manufacture. Microbial bioprocesses show great potential for the sustainable bioproduction of these essential commodities. For example, gas-fermenting bacteria such as acetogens produce acids and solvents such as acetate or ethanol while simultaneously reducing greenhouse gases through autotrophic conversion of $CO_2$ and CO using the Wood-Ljungdahl pathway. The thermophilic acetogen *Moorella thermoacetica* has additional advantages in an industrial setting, due mainly to its ability to grow at high temperature. However, genetic manipulation of this organism remains challenging, preventing its metabolic engineering for industrial purposes.

Transformation of *M. thermoacetica* requires the circumvention of several physical and biochemical barriers such as restriction-modification systems. A key step towards more efficient transformant generation relies on creating an autonomously replicating shuttle vector in *M. thermoacetica*. Plasmid replication requires that the plasmid carries a compatible Gram-positive replicon. Such a replicon from another thermophilic organism has already been identified in this research project. Although this progress allows a subpopulation to carry the plasmid, mixed populations remain problematic, and different avenues are currently explored to obtain pure mutant cultures. In addition, a reporter system to assess promoter strength is currently being developed for *M. thermoacetica.*

## P131

# Genetic manipulation of Lactobacillus to produce optically pure lactic acid following fermentation using hydrolysed organic fraction of municipal solid waste as a feedstock

Christopher Letchford-Jones[1], David Bryant[1], Alun Hughes[1], Dhivya Puri[2], Joe Gallagher[1]
[1]Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, United Kingdom. [2]Fiberight Ltd. Unit 73 Basepoint Enterprise Centre, Southampton, United Kingdom

## Abstract

Background

Lactic acid for polymer applications must be optically pure to allow the final plastic properties to be controlled. We have isolated a strain of Lactobacillus plantarum capable of utilising a range of carbohydrates and tolerant to high sugar and organic acid concentrations. However, like most Lactobacillus plantarum, this strain produces D- and L-lactic acid. To improve the commercial application of this strain gene editing has been used to ensure only one isoform is produced.

Methods

Using two-step homologous recombination, ldhL was replaced with a truncated version interrupted by a chloramphenicol acetyltransferase resistance marker, then with an unmarked truncated version.

The optical purity shift was quantified biochemically by enzymatic assay and HPLC and fermentation performance compared against the unmodified strain using enzymatically hydrolysed municipal solid waste pulp (MSW sugars) as a feedstock.

Results

The ldhL gene was successfully deleted, leading to a dramatic shift in the optical purity of lactate produced in both synthetic media and MSW sugars. The unmodified strain produced D-lactic acid with an optical purity of 40%, while the mutant achieved 94%. In MSW sugars, the modified strain performed as well as the unmodified control, consuming all available glucose in less than 48 hours with a volumetric productivity of 1.02 g/L/h.

Conclusions

Deletion of ldhL from our strain of Lactobacillus plantarum allowed exclusive production of D-lactic acid from MSW sugars. This provides a laboratory scale demonstration of the production of optically pure lactic acid from renewable sugars for use in the production of biodegradable plastics.

## P132

# High yield chemostat production of nanomagnets by a marine magnetotactic vibrio

Tarcisio Correa[1], Mateus Godoy[1], Dennis Bazylinski[2], Fernanda Abreu[1]
[1]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. [2]University of Nevada, Las Vegas, Las Vegas, USA

## Abstract

Magnetotactic bacteria (MTB) are the main source of biogenic magnetic nanoparticles (BMNs). BMNs are membrane-enveloped magnetic crystals with diverse applicability in biotechnology. Nevertheless, the large-scale cultivation of MTB remains challenging due to relatively low yields and improved cultivation methods are necessary. In this work, we have established continuous cultivation of *Magnetovibrio blakemorei* strain MV-1$^T$ in a 2-L benchtop bioreactor. The magnetotactic vibrio strain produces prismatic magnetite BMNs with an approximate mean diameter of 60 nm. Preliminarily, a fed-batch cultivation with iron source supplementation and 24-h spaced injections of nitrous oxide was developed. In this strategy, a magnetite yield of 24.5 mg/L was reached after 120 h, with peak productivity (16.8 mg/L/day) occurring between 48 and 72 h. A reduction in the production of magnetosomes per cell was observed by transmission electron microscopy from 72 h. To prevent the loss of magnetosome production, we designed continuous cultivation in a chemostat mode. The steady state was established using an intermittent nitrous oxide injection strategy coupled with agitation control at 8-h intervals. During chemostat cultivation, a peak yield of 27.1 mg/L and productivity of 22.7 mg/L/day were observed at 120 h, as well as the maintenance of similar values until 168 h. Chemostat cultivation of MTB is advantageous because it allows a longer cultivation period with cells producing BMNs in high intensity.

## P132

# Powering Efflux - Deciphering the Energetics of Bacterial Tolerance to Platform Chemicals of the Plastics Industry to Enhance Sustainable Manufacturing

Eilidh Terras, Nicholas Tucker, Arnaud Javelle
University of Strathclyde, Glasgow, United Kingdom

## Abstract

*Pseudomonas spp.* are known for their multidrug resistance and solvent tolerance conferred through several highly efficient efflux systems. The *Pseudomonas* tripartite RND efflux pumps are integral in pathogenicity and their expression is often increased in clinical isolates. Increasing global demand for high-clarity and high tensile strength plastic has led to industrial interest in a fermentation process for the production of monomers from non-petrochemical sources. This requires a bacterial strain with high tolerance to these chemicals, particularly solvents. Therefore *Pseudomonas* spp. are promising candidates for this fermentation, and the role of efflux in solvent tolerance is crucial to successful process development. Transposon insertion mutants of RND pumps and transporters associated with various efflux systems show increased sensitivity to industrial solvents compared to the wild type. We demonstrate the purification of a variety of efflux pumps we believe are essential for solvent tolerance in *Pseudomonas aeruginosa*. The functional relationship between these systems is investigated using solid supported membrane electrophysiology (SSME) to determine the transport activity of several solvents by these proteins. Phenotypic microarray studies also show that our transposon mutants of these systems behave similarly in the presence of a range of solvents, strongly suggesting a functional relationship. Understanding the relationship between these systems will not only aid in the development of plastic production strains but may also provide a new drug target for drug resistant *Pseudomonas* strains.

## P133

# The endophytic *Pantoea agglomerans* 33.1 as a RNAi-delivery vector to control insect pests

Bruna Factor[1], Dyson Paul[2], Maria Verdi[1], Antonio Figueira[1]
[1]University of São Paulo, Piracicaba, Brazil. [2]University of Swansea, Swansea, United Kingdom

## Abstract

RNAi has been proposed as an alternative to mitigate the impact of insect pests in agriculture by an exposure to dsRNAs, homologous to an essential insect gene. One possible dsRNA delivery method includes insects feeding on a diet containing microorganisms expressing dsRNA. This method has some advantages such as large-scale production, lower cost and protection against degradation caused by insect midgut alkaline pH and nucleases activity. Successful microorganism-mediated RNAi depends on the lack of an active RNase III, encoded by *rnc* gene in these organisms. RNase III is crucial for processing various types of RNAs but is also characterized by degrading dsRNAs. Therefore, to develop a plant endophytic bacterium expressing dsRNA targeting sugarcane pests as control method, we knocked the RNase III *rnc* down from *Pantoea agglomerans* 33.1. This species is a Gram-negative Enterobacteriaceae bacterium, frequently found associated with a wide range of plant species, colonizing leaves, stems and roots. This colonization brings benefits to the hosts, since *P. agglomerans* 33.1 contributes to the promotion of plant growth, either by the production of indole-acetic acid, phosphate solubilization or nitrogen fixation. We identified and characterized *rnc* of *P. agglomerans* 33.1, found colonizing sugarcane. Subsequently, we edited the *rnc* gene by λ-red homologous recombination to insert the kanamycin resistance gene into the *P. agglomerans* 33.1 *rnc*. Transformants of *P. agglomerans* 33.1 cells were successfully obtained with the edited *rnc*. We demonstrated that this edited bacterium is viable and able to colonize sugarcane tissues. This *P. agglomerans* 33.1 D-rnc will now be used to deliver dsRNA against pests.

## P134

# Understanding the bug that make our drugs: Cellular responses to therapeutic protein secretion in *E. coli*.

Alice Seleiro[1], Caroline Evans[1], Jeff Green[1], Chris Lennon[2], Graham Stafford[1]
[1]University of Sheffield, Sheffield, United Kingdom. [2]FUJIFILM diosynth biotechnologies, Billingham, United Kingdom

## Abstract

Production of therapeutic proteins is a $200 billion industry with *E. coli* being one of the main expression systems used and proteins often being secreted to the periplasm to produce disulphide bonds. However, little is known about how *E. coli* responds to secretion of these therapeutic proteins into the periplasm under industrial conditions.

Proteomics and transcriptomics were conducted on cultures grown at high cell density in industrial production conditions to look at the effect of secreting a model therapeutic protein (scFv) to the periplasm compared to expressing the same protein in the cytoplasm.

82 proteins and 1653 transcripts were differentially expressed when overexpressing the scFv in different cellular compartments. ScFv secretion had a significant effect on expression of genes involved in the envelope stress response. However, while certain envelope stress pathways where upregulated upon secretion others were downregulated (including expression of some periplasmic chaperones). Scfv secretion also led to an increase in expression of genes involved in degradation of membrane proteins and the SEC secretion system, as well as iron transport, Outer membrane porins and flagella operons.

In future and current experiments, we are testing whether knocking out and overexpressing a selection of these genes improves secretion and folding of scFvs and other recombinant proteins.

## P136

# Perfluorooctanoic acid (PFOA) sensitises *E. coli* to acid stress

Gaurav Chugh[1], Conor P O'Byrne[2], Florence Abram[1]
[1]Functional Environmental Microbiology laboratory, Microbiology, Ryan institute, National University of Ireland Galway, Galway, Ireland. [2]Bacterial Stress Response laboratory, Microbiology, Ryan Institute, National University of Ireland Galway, Galway, Ireland

## Abstract

Per-and Polyfluoroalkyl Substances (PFAS), which includes PFOS and PFOA, are recognized as the most important class of emerging contaminants due to their widespread presence in wildlife and humans, their environmental persistence, bioaccumulative potential, and toxicity. Low environmental pH conditions affect the state and physicochemical properties of PFAS, which in turn impacts on their toxicity. Sparse information is available regarding the effect of these compounds on microorganisms, and the possible associated knock-on effect on ecosystem services. The purpose of this study is to investigate the ability of *E. coli* to tolerate stress in the presence of PFAS compounds. To this end, we carried out phenotypic comparisons of *E. coli* exposed to a series of environmental conditions in the presence and absence of PFOA and PFOS. Quite remarkably, *E. coli* growth was not affected by the presence of PFOA and PFOS up to 500 mg/L. The survival of *E. coli* at pH3, however, decreased by more than three-fold when the medium was supplemented with PFOA. PFOA and PFOS were also found to decrease the growth rates of *E. coli* in minimal media in the presence of 0.75 and 1M NaCl. We are currently screening further stresses of environmental relevance with the aim to conduct molecular investigations to examine the mechanisms underpinning the effect of PFAS exposure on bacterial stress tolerance. This work will provide some insights into the impact of PFAS on microorganisms, which should shed new light on the assessment of the ecological effects of PFAS.

## P137

# Assessing the anti-biofilm potential of bacteria with presumptive AI-2 quorum quenching activity using a polymicrobial *in vitro* model

Amy Kate Falà[1,2,3], Cormac G. Gahan[2,1], Avelino Alvarez-Ordóñez[4,5], Alain Filloux[6], Paul D. Cotter[3,1]
[1]APC Microbiome Ireland, Cork, Ireland. [2]School of Microbiology, University College Cork, Cork, Ireland. [3]Teagasc Food Research Centre, Fermoy, Ireland. [4]Department of Food Hygiene and Technology, Universidad de León, León, Spain. [5]Institute of Food Science and Technology, Universidad de León, León, Spain. [6]MRC Centre for Molecular Bacteriology and Infection, Department of Life Sciences, Imperial College London, London, United Kingdom

## Abstract

**Background**

Biofilms allow bacterial consortia to live attached to surfaces, with increased protection by the EPS matrix and enhanced cell-cell communication due to enforced proximity. Biofilm formation is facilitated in part by quorum sensing processes between micro-organisms and, thus, inhibition of this communication could find applications in prevention of biofilm formation or facilitation of destruction through enhanced antimicrobial sensitivity.

**Methods**

A biobank of ~3000 bacterial isolates was screened for inhibition of AI-2-mediated quorum sensing using the indicator organism *Vibrio harveyi* DSM19623. Presumptive positive isolates were evaluated for their anti-biofilm potential using a polymicrobial static *in vitro* assay (*Cronobacter sakazaki*, *Listeria monocytogenes, Pseudomonas fluorescen*) through the application of neutralised cell free supernatants (CFS), with biomass formed measured by crystal violet staining. Biofilm inhibitory potential was evaluated by incubating CFS with log cultures of the biofilm-forming strains, while biofilm dispersal potential was examined by incubating CFS with 72 hour-old preformed biofilms.

**Results**

Over 200 isolates from the bank of 3000 bacterial strains were observed to produce presumptive inhibition of AI-2 quorum sensing, assessed by measuring the degree of inhibition of bioluminescence production by *V. harveyi* DSM 19623 without growth inhibition of the indicator strain. 73 of these isolates were found to inhibit biofilm formation, while 23 isolates appeared to promote dispersal of mature biofilm.

## Conclusion

Quorum sensing is implicated in biofilm formation and this study supports its targeting for the development of novel antimicrobial therapies. The most promising isolates are being identified by 16S rRNA sequencing and characterized using a functional genomics approach.

## P138

# Optimization of Fermenter Cultural Conditions for *Brucella abortus* strain 99.

saeed alamian[1], somaye mahmoudpour[2], afshar etemadi[1], morteza taghizadeh[1]
[1]Department of Brucellosis, Razi Vaccine and Serum Research Institute. Agricultural, Research Education and Extension Organization (AREEO), Karaj, Iran, karaj, Iran, Islamic Republic of. [2]islamic azad university of tehran north branch, karaj, Iran, Islamic Republic of

## Abstract

Introduction:Brucellosis is one of the bacterial zoonotic diseases caused by Brucella species and its occurrence in humans and animals leads to severe health and economic loses. To prevent and control this disease, we need to mass-produce diagnostic antigens and vaccines. *B.abortus S99* used as the reference strain for antigen production. Culture in a solid medium in the flask and fermenter are two common methods for producing Brucella antigen. In this study, we investigated the optimization of culture method in the fermenter.

Methods:Seed culture prepared from the lyophilized *Brucella abortus strain S99*. we used the Brucella broth medium, as a base culture media. The medium pH and temperature adjusted at 6.8 and 37 ° C, respectively. The agitation speed varied from 400 to 750 rpm during 48 hours. The airflow rate was set between 8-4 min / L-1 to maintain the percentage of dissolved oxygen at 25% -30%. Sampling performed at each stage and the samples examined for cell density and morphological conditions of the bacteria. The culture process completed after 48 hours and the product harvested.

Results : The results showed that the yield of this method was $90x10^9$ CFU after 48 hours of cultivation

Conclusion : Antigen production based on the conventional method is not efficient, so fermenter method can be considered in industrial production of brucellosis diagnostic antigen. According to the results of this study, Cell density, purity and efficiency of the antigen produced are acceptable. Therefore, we can use this method to mass-produce antigens instead of existing methods.

## P139

# Immunogenicity assessment of Clostridium perfringens TOXID with Inactivated Type O foot-and-mouth Virus in Animal Model

morteza taghizadeh[1], arezou araghi[2], alireza pardis[3], SEID MAHMOOD AZIMI[1], Reza Hosseini Doust[2]
[1]Razi vaccine and serume institute, karaj, Iran, Islamic Republic of. [2]Microbiology Department, Medical Sciences University Islamic Azad (MSUIA), Tehran, Iran, Islamic Republic of. [3]RAZI VACCINE AND SERUME INSTITUTE, karaj, Iran, Islamic Republic of

## Abstract

**Foot-and-mouth disease (FMD) and enterotoxemia are a major infectious disease in livestock. Toxoid vaccine can provide protective immunity against enterotoxemia disease. since the duration of the toxoid vaccine and Inactivated FMD immunogenicity is short, these vaccines need to contain an adjuvant. the choice of adjuvant is a very important factor in enhancing immune responses and the efficacy of inactivated vaccines. nanoparticles of chitosan,ISA206 can stimulate humoral and cell Mediated immune responses than aluminum hydroxide gel and mf59 adjuvant. in study the new protocol is A proprietary adjuvant system was used to formulate the vaccines that conferred effective protection at low doses while maintaining the DIVA compatibility**.

**Methods:**

**Clostridium perfringens Type C-D-B Growth**

**Purification of beta-toxin (type:B-C),and epsilon (type:D)**

**Determination of protein concentration**

**LD50 determination**

**Preparation of virus vaccines: virus was suspension BHK21 cell culture**

**Centrifuged and suspension+8% polyethylene glycol 6000. titer by the TCID50 and inactivated by 4 m/M binary ethyleneimine.**

**Experiment:**

**1-vaccine mixing the 50% ISA206**

**2-vaccine mixing the 30% aluminum hydroxide**

**3-vaccine mixing the chitosan**

**Injection into the animal**

**- Antibody assay**

**- Potency test**

**Results:**

**we showed Evaluation of Toxoid vaccine and Inactivated vaccine with adjuvant chitosan or ISA 206 was safe and elicited no detectable immunoglobulin (IgE) response in sheep and goat.**

**Conclusion:**

**In this study showed ISA 206,chitosan with high-potential immune responses need for clinical protection against FMD and Clostridium perfringens infection.we believe that this new formulation has great potential for use as an adjuvant in livestock vaccines**

## P140

# Living paints: Encapsulating metabolically active bacteria in biocoatings

Simone Krings, Yuxiu Chen, Mira Sulonen, Claudio Avignone Rossa, Joseph L. Keddie, Suzanne Hingley-Wilson
University of Surrey, Guildford, United Kingdom

## Abstract

Background

Biocoatings combine colloidal polymers with metabolically active bacteria. They are "living paints", displaying high porosity, which allows gaseous exchange and enhanced bacterial hydration.

Methods

The model organism *E. coli* or the marine or freshwater cyanobacterial strains (*Synechococcus* sp. PCC 7002 and *Synechocystis* sp. PCC 6803, respectively) were mixed with aqueous polymer nanoparticles (i.e. synthetic latex) and inorganic nanotubes (halloysite) and their toxicity was evaluated by colony-forming units (CFU) and resazurin assays. Suspensions were cast onto poly-L-lysine-coated glass slides and dried to achieve coalesced biocoatings. The viability of the bacteria within these biocoatings was then assessed by confocal laser scanning microscopy (CLSM) and resazurin assays.

Results

Several concentrations of halloysite were tested to find the optimal permeability of the coatings. Higher permeability of liquid was found in biocoatings containing 29 wt% halloysite. Analysis of the biocoating microstructure, through scanning electron microscopy of freeze-dried samples, revealed bacterial encapsulation and a surrounding network of pores.

Using CLSM, viable cells were tracked by chlorophyll *a* for cyanobacteria and constitutively expressed yellow-fluorescent protein for *E. coli*, whereas dead cells were tracked by secondary pigments and the membrane exclusion dye propidium iodide, respectively. Subsequent analysis by ImageJ revealed higher proportions of viable bacteria within halloysite biocoatings.

Conclusions

We assessed the viability in the suspensions and the highly porous biocoatings, which could be used as oxygenating films with species of the Cyanobacteria phylum. The use of other bacterial species, such as acetogenic or genetically modified bacteria, could generate several applications, including bioremediation and sustainable energy production.

## P140

# Polyesterase activities in bacterial isolates from seaweed and sponges, with potential utility in polyethylene terephthalate plastic and nanoparticle hydrolysis.

Clodagh M. Carr[1], David J. Clarke[1], Alan D. W. Dobson[1,2]
[1]School of Microbiology, University College Cork, Cork, Ireland. [2]SSPC-SFI Research Centre for Pharmaceuticals, University College Cork, Cork, Ireland

## Abstract

Many marine bacteria have evolved to produce a range of extracellular enzymes which facilitate their growth and survival in the harsh, oligotrophic conditions often present in marine environments. Marine sponge derived *Streptomyces* strains have previously been reported to produce polyesterase enzymes, which are of interest for several biotechnological applications, including polyethylene terephthalate (PET) plastic hydrolysis.

Bacteria isolated from sponges and seaweed were screened for polyester hydrolysis activities using plate-clearing assays. Lipolytic and polyesterolytic activities were initially identified by employing tributyrin and polycaprolactone diol agar-based assay systems, respectively. Polyesterase activity was subsequently confirmed on both polycaprolactone and on PET-nanoparticle agar plates, resulting in the prioritisation of six isolates for Illumina next-generation genome sequencing.

These include three *Bacillus* spp., isolated from the brown seaweed *Ascophyllum nodosum,* and from marine lake sponges *Stelligera stuposa* and *Eurypon major*, together with a *Maribacter* strain again isolated from *S. stuposa,* and *Brachybacterium* sp. and *Micrococcus* sp. isolates of deep-sea sponges *Pheronema* sp. and *Inflatella pellicula*, that were sampled at depths of 2129m and 2900m, respectively. Genome mining and comparative genomic analysis of these isolates is currently underway to identify genes encoding the observed activities and to assess homology with known PET hydrolases.

Microbes found living in association with filter-feeding sponges may have increased exposure to the plastics and microplastics that widely contaminate our marine ecosystems, thus representing a promising source of degradative activities towards synthetic polymers that could contribute to new plastic waste management strategies.

**P141**

# Developing novel pathogen biocontrol using plant-derived allyl-ITC allelochemical

Carrie Alderley
University of York, York, United Kingdom

## Abstract

Crop losses to plant pathogens are a growing threat to global food security. Biofumigation is a promising agricultural biocontrol technique, which harnesses antimicrobial plant allelochemicals, such as isothiocyanates (ITCs) derived from *Brassica,* to protect crops against disease. Here we used several laboratory models to develop an ITC-based biocontrol method against *Ralstonia solanacearum* plant bacterial pathogen: the causative agent of bacterial wilt. We first identified allyl-ITC as a highly suppressive antimicrobial that limited the growth of *R. solanacearum* in lab media. We then validated its efficacy in soil microcosms and found that *R. solanacearum* is relatively more susceptible to allyl-ITC compared to *Pseudomonas* plant growth-promoting bacteria. Moreover, 16S rRNA amplicon sequencing revealed that while no significant change in soil microbial community diversity was observed, allyl-ITC application led to changes in bacterial community composition, specifically in enrichment of *Actinobacteria*. Importantly, no harmful effects were observed on plant biomass or chlorophyll contents, and weekly allyl-ITC application could protect 57% of tomato plants from bacterial wilt. Finally, we tested if repeated exposure to allyl-ITC could select for improved tolerance using experimental evolution. Parallel mutations were observed in a third of the replicates, which were associated with potential ITC breakdown (dehydrogenases) and cell wall structure (serine/threonine kinases). However, no tolerance evolution was observed in soil microcosms. Together, these results suggest that allyl-ITC is a promising plant-derived antimicrobial, which could potentially be used to control bacterial wilt disease without collateral damage to beneficial rhizosphere microbiota.

**P141**

# 4000 metres closer to novel antibiotics: a bioprospecting tale of three *Streptomyces* strains from the Andes

L. Pintor-Escobar[1], M.M. Zambrano[2], L.T. Fernández-Martínez[1]
[1]Edge Hill University, Ormskirk, United Kingdom. [2]Corporación Corpogen, Bogotá, Colombia

## Abstract

Bioprospecting of underexplored environments and microbiomes remains one of the core strategies for drug discovery. Páramos, a high-altitude ecosystem and evolutionary hotspot in the northern Andes of South America, harbour microbial diversity yet to be studied for its potential for antibiotic production. In this project, three strains (CG885, CG893 and CG926) isolated from a páramo in Colombia were explored using phylogenetic and genome mining tools to uncover their potential for novel antimicrobials. Taxonomic characterisation of three isolates suggested strains CG885 and CG893 can be classified as *Streptomyces pratensis* while strain CG926 is likely to be a new *Streptomyces* species. All three strains showed the biosynthetic coding capacity characteristic of streptomycetes and a diverse repertoire of biosynthetic gene cluster types likely to encode for novel specialised metabolites. One cluster in the strain CG926, for instance, is predicted to encode for a new halogenated compound containing the unusual nonproteinogenic amino acid piperazic acid with no known analogue. Integration of these results with phenotypic and metabolomic data will enable the assessment of these molecules and their antimicrobial activity. Overall, these results demonstrated that these three strains from an underexplored environment harbour the potential to become producers of novel antibiotics.

## P142

# Evaluation of the fast-growing bacterium Vmax as a production host for heterologous outer membrane proteins.

Jack C Leo, Sree Harshika Thota
Nottingham Trent University, Nottingham, United Kingdom

## Abstract

Gram negative bacteria usually have 20–30% of all genes encode membrane proteins. There are two classes of membrane proteins: β-barrel membrane proteins and helical bundle membrane proteins. Beta-barrel transmembrane proteins are exclusively found in the outer membrane. These outer membrane proteins (OMPs) have a wide range of functions from nutrient intake and protein secretion to biofilm formation. They are also potential drug and vaccine targets. The usual system for over-expression of OMPs is *Escherichia coli.* However, heterologous OMPs are not always well expressed in *E. coli* due to inefficient recognition by the BAM complex. This can lead to misfolding of the proteins and additional stress on the cells, further reducing growth rate and protein yield.

In the present study we are evaluating the fast growing Vmax strain of *Vibrio natriegens* as an alternative host for heterologous OMP production. As a first step in evaluating the potential of *V. natriegens*, we performed a bioinformatic analysis to identify the endogenous OMPs using online tools such as BLAST, BOMP, HHOmp and subcellular localization prediction programs. This was done to identify candidates for gene deletion to improve the yield of OMPs. In addition, the signal peptides of the endogenous OMPs were determined as they could be used to enhance the production of heterologous OMPs. The next step will be to produce several heterologous OMPs in Vmax and determine correct outer membrane integration. We will then compare production efficiencies with *E. coli.*

**P143**

# Improving production of chaplin proteins from Streptomyces for the development of sustainable protein-based corrosion-resistant coatings

Craig Allan, Gethin Allen, Alex Harold, Sholeem Griffin, David Penney, Geertje van Keulen
Swansea University, Swansea, United Kingdom

## Abstract

We aim to further develop protein-based technology for nanometers-thin coatings for improved corrosion resistance and adhesion to metal as a potential substitute for Chromium VI. The non-pathogenic functional bacterial amyloids chaplins (coelicolor hydrophobic aerial proteins, Chp) produced by *Streptomyces,* during morphological differentiation only, have been conceptually proven to provide corrosion resistance to metals. Chaplins also confer strong adhesive properties to hydrophilic non-metal substrates when applied as a composite.

Protein production levels and cost of fermentation are currently limiting maturation of technology and scale-up. For instance, the most expensive media component for fermentation is TES (~£1000/Kg), while the complex composition of R2YE media further exacerbates cost and ease of upscaling.

Approaches taken in this study to optimise the (cheaper) production of chaplin proteins include: (i) inducible overexpression of (modified) chaplin protein(s) in a homologous host using a thiostrepton-inducible promotor plasmid for the expression of a single chaplin gene; and (ii) altering media composition and fermentation conditions for improve chaplin expression and yield. Preliminary results shows visual uptake of Congo red dye from the media suggesting ChpE expression occurs.

Alternative buffers and simpler media compositions were trialled upon which protein production was compared to TES-buffered R2YE media via an amyloid-specific dye, Thioflavin T- and mass spectrometry-based assays. Sufficient biomass generation could be realised upon media simplification, however, chaplins could only be produced upon addition of one alternative buffer only. With these changes in media and buffer, significant cost reductions could be gained and thus allowing for improved scalability of chaplin production.

**P144**

# Sorghum beer production using *Saccharomyces sp*. isolated from palm wine as the pitching yeast and *Garcinia kola* as the bittering agent

Tochukwu Nwagu, OnyetugoChioma Amadi, Bassey Okon, Anastesia Ozioko, Chukwudi Nnamchi, Anene Moneke, Bartholomew Okolo, Reginald Agu, Ifeany Ndubuisi
University of Nigeria, Nsukka, Nigeria

## Abstract

Beer production in Nigeria is dependent on imported brewery yeasts and hops, which consequently increases the cost of beer production. The current study was conducted to determine the effect of using indigenous strains of *Saccharomyces* sp. in place of brewer's yeast, and Garcinia kola as the bittering agent on beer quality. *Saccharomyces* species strains were isolated from palm wine, and *Saccharomyces* cerevisiae specie selected using nitrates and lysine assimilation test. Indigenous *S. cerevisiae* was used for beer brewing at different pitching rates ($1\times10^7$, $2\times10^7$, & $3\times10^7$) using brewer's yeast as the control. The effect of various concentrations of *Garcinia kola* (0.1 ml, 0.2 ml and 0.3 ml) on beer taste and quality was later determined; beer brewed with hops served as the control sample. The physicochemical analysis showed that the pH, specific gravity (SG) and free amino nitrogen (FAN) values of beer brewed with local *S. cerevisiae* strain were between 4.32 - 4.52, 0.962 - 1.008, and 81.80 - 263.64 g/L, respectively while the alcohol value was from 5.12 - 11.16 ABV. However, pitching with 1×107 cells gave the best product and was therefore used for further analysis. Beer samples from wort pitched with local yeasts and brewed with *Garcinia kola* gave good quality beer comparable to the standard product. pH values of the products were in the range of 3.18 - 4.41, SG (0.962 - 1.006), FAN (16.87 - 81.80g/l), bitterness (8.83 - 17.83 EBU) and alcohol (5.38 - 11.16 ABV).

**P146**

# Diversity Analysis and Genome Mining of Microbial 16S rRNA Gene and Metagenomic Data for the Discovery of Novel Antibiotics

Josephine Giard[1], Jennifer Pratscher[2], Leena Kerr[1]
[1]Institute of Life and Earth Sciences, School of Energy, Geoscience, Infrastructure and Society, Heriot-Watt University, Edinburgh, United Kingdom. [2]The Lyell Centre, Heriot-Watt University, Edinburgh, United Kingdom

## Abstract

There is an urgent need for new antimicrobials due to constantly advancing antimicrobial resistance. Here, we worked with environmental samples from diverse habitats including different savannah and forest soils, volcanic caves, and termite mounds and assessed their microbial communities for the potential of biosynthesis of secondary metabolites. We analysed and compared microbial composition by applying the QIIME2 pipeline to 16S rRNA gene data. We focused on the abundance of Actinobacteria and *Streptomyces* as they are important producers of antimicrobials. Out of the samples analysed, the highest abundance of Actinobacteria was found in termite mound and volcanic cave samples. Moreover, the termite mound samples also had the highest abundance of *Streptomyces*. When comparing microbial composition, soil samples and termite mound samples each formed their own clusters, but volcanic cave samples appeared more dispersed. We assessed the antimicrobial potential of a subset of samples by analysing metagenomic data to predict biosynthetic gene clusters (BGCs) using antiSMASH5.2.0, which resulted in over 800 hits per sample. This number was narrowed down by evaluating identified BGCs based on antimicrobial potential, completeness, size, presence/absence of regulatory and transport-related genes, and dissimilarity with known BGCs. This resulted in an average of 20 BGCs per sample. These BGCs will be subjected to further sequence-based analyses before attempting heterologous expression. Following successful expression, antimicrobial potential will be assessed by screening for growth inhibition of multidrug resistant *E.coli* strains and the ESKAPE pathogens.

**P147**

# Engineered hybrid MerR transcription factors enable construction of novel MerR based synthetic circuits for heavy metal detection in *Bacillus subtilis*

Jazz Ghataora, Susanne Gebhard, Bianca Reeksting
University of Bath, Bath, United Kingdom

## Abstract

Anthropogenic activity including burning of fossil fuels, overuse of pesticides and fertilisers and the release of contaminated industrial effluent has substantially increased toxic heavy metal deposition into the environment. Conventional analytical methods for monitoring heavy metal pollution are hampered by large equipment costs, the requirement for trained personnel, and inability to quantify heavy metal bioavailability. Bacterial biosensors offer a promising alternative to these methods. The well-characterised genetics of *Bacillus subtilis* and its ability to produce spores for long-term storage make it an ideal choice for use in biosensor development. Although *B. subtilis* possesses innate metal detection systems, different mechanisms of transcriptional regulation, variation in metal specificity and sensitivity impedes flexible synthetic biosensor circuit design for metal detection. By contrast, metal-sensitive MerR family regulators possess a conserved and tightly controlled mechanism of transcriptional regulation with both high sensitivity of detection and metal specificity. However, as the majority of diversity in metal specificity in MerR is found in Gram-negative species, species-specific differences in biological part usage limits their implementation in Gram-positive species, such as *B. subtilis.* To overcome this design constraint, a $Hg^{2+}$-specific MerR system from *Staphylococcus aureus* was modified to produce a novel $Zn^{2+}$-sensing hybrid MerR regulator by fusion of the MerR DNA-binding domain with the metal-binding domain from the $Zn^{2+}$-responsive MerR homologue ZntR. This yielded a functional hybrid (MerRZntR) with preferential detection for $Zn^{2+}$ ($Zn^{2+}$>$Hg^{2+}$). This work demonstrates a potentially flexible approach to design modular metal detection circuits using additional MerR hybrids based on Gram-negative systems for use in *B. subtilis* biosensor development.

## P148

# Engineering a microbial 'trap and release' mechanism for microplastics removal

YANG LIU
The Hong Kong Polytechnic University, Hong Kong, Hong Kong

## Abstract

Microplastics are currently accumulating in the environment as pollution, so it has been intensively recognized as a major environmental concern now. Due to the difficulty in collection and digestion of microplastics, the particles can accumulate in food chains within aquatic bodies, ultimately influencing human health. In terms of the treatment of microplastic pollutants, methods utilized currently, like incineration or landfill, have different disadvantages, and among these methods, recycling is a better choice with a potential prospect. Other than these methods, bioremediation strategies possess the potential to remove microplastic pollution. As proof-of-concept, we employed a ubiquitous environmental bacterium, *Pseudomonas aeruginosa*, to form biofilms and trap microplastics together. After subsequent processing, the microplastic particles can be removed from the bacterial biofilm and release from the matrix for recovery. The *Pseudomonas aeruginosa* biofilm with a trap-and-release mechanism used its sticky exopolymeric substances (EPS) to bioaggregate microplastics for easier isolation. Its biofilm could be dispersed to release the captured microplastics for convenient collection and recycling. We also demonstrated the potential application of the engineered biofilm in removing microplastics pollution in marine water samples collected in nature near a sewage outfall. Hence, our engineered bacterium can accumulate microplastic particles of various sizes and materials in its biofilm EPS, where our capture-and-release mechanism provides a basis for the future use of bacterial biofilm to capture and recycle microplastics.

**P149**

# Removing acetate formation and maximizing citramalic acid production by metabolically engineered *Escherichia coli.*

Giorgio Jansen[1], Giuseppe Nicosia[2], Stephen Oliver[1]
[1]University of Cambridge, Cambridge, United Kingdom. [2]University of Catania, Catania, Italy

## Abstract

Fermentation by genetically engineered microbes has potential for the sustainable production of methacrylic acid, used in the manufacture of Perspex®. We have used metabolic modelling techniques to examine citramalic acid production in *Escherichia coli* expressing the citramalate synthase gene *cim*A from *Methanococcus jannaschii* in order to convert acetyl-CoA and pyruvate to citramalate. In our simulations, we used the iML1515 *E. coli* genome-scale metabolic model, Lund medium, and constraints on gene deletions based on both essentiality and synthetic lethality. In this way, we investigated possible genetic manipulations to maximise the production of citramalate and eliminate that of the undesirable by-product, acetate.

This methodology permitted the design of a population of strains with the desired characteristics. Analysis of these strains identified 4 classes of gene deletion: (I) those that eliminated acetate production, (II) those maximising citramalate production, and (III) those that ensured a basic level of citramalate production (making these strains particularly robust and useful in industrial applications), and (IV) those that achieved the dual objective of maximising citramalate production while preventing that of acetate. Finally, our simulations predicted that an *E. coli* strain with seven gene deletions would maintain an acceptable growth rate, just 30% less than wild-type *E. coli,* while eliminating acetate formation and more than doubling the wild type's productivity of citramalate.

## P150

# Optimisation of poly-(gamma glutamic acid) (γ-PGA) biosynthesis from *Bacillus subtilis* natto using waste algal fractions

Mattia Parati[1], Ibrahim Khalil[1], Fideline Tchuenbou-Magaia[2], Barbara Mendrek[3], Grazyna Adamus[3], Izabela Radecka[1]
[1]Wolverhampton School of Sciences, Faculty of Science and Engineering, University of Wolverhampton, Wolverhampton WV1 1LY, UK, Wolverhampton, United Kingdom. [2]Wolverhampton School of Engineering, Faculty of Science and Engineering, University of Wolverhampton, Wolverhampton WV1 1LY, UK, Wolverhampton, United Kingdom. [3]Centre of Polymer and Carbon Materials, Polish Academy of Sciences, 41-800 Zabrze, Poland, Zabrze, Poland

### Abstract

Poly(gamma-glutamic acid) (γ-PGA) is an anionic, and biodegradable, pseudopolypeptide extracellularly synthesised by several *Bacillus* species. γ-PGA can present diverse characteristics based on the ratio of D, L or D/L acid residues in the polymeric chain, the molecular mass (Mw) and its form (free acid form or salt form). This variety in material's properties enables it to satisfy an array of sector-specific applications, ranging from bioremediation to medical materials. However, the production of this biopolymer is still very expensive as it requires costly substrate components.

The aim of this study was to determine whether waste algal fractions could be employed for the biosynthesis of γ-PGA, and if these could improve production economics. All small-scale shake-flasks experiments (250 mL volume) were performed under aerobic conditions (150 rpm). *Bacillus subtilis* natto ATCC 15245 cells were grown at 37$^{O}$C for 96hrs. To monitor microbial growth, samples were withdrawn at 24hrs intervals. Samples were serially diluted, plated on TSA and incubated overnight at 37$^{o}$C. Ultimately, cell numbers were counted and results expressed as CFU ml$^{-1}$.

We investigated the effects of medium components (glutamic acid, sugars and algal fraction) on γ-PGA production and microbial growth (CFU ml$^{-1}$). Further optimisation of downstream processing was carried out by substituting ethanol precipitation with copper sulphate precipitation. Isolated polymer was identified using Fourier-Transform Infrared Spectroscopy (FTIR). Overall, the productivity of the γ-PGA polymer was comparable to that of expensive defined media (GS media) and therefore the use of algal waste could help facilitate the economics of γ-PGA biosynthesis.

**P151**

# Promoting hydrolysis of African star apple peel by yeast for cellulase, pectinase production and stabilization

Onyetugo Amadi, Ifeanyi Ndubuisi, Jane Udeh
University of Nigeria, Nsukka, Nigeria

## Abstract

Enzyme industry are constantly seeking enzymes with more robust abilities. Pectinolytic and cellulolytic enzymes are widely used in the food industry for juice and wine production. This work was carried out to determine the potential of yeast isolate in producing pectinase and cellulase from African star apple peels. Culture parameters for enzyme production were optimized. Enzymes were partially purified, characterized and stabilization studies were also carried out. Fermentation results indicate that the peak of the enzyme synthesis from the peels was at 96h of fermentation with cellulase activity of 72.1U/ml and pectinase activity of 35U/ml. Ammonium sulphate at 70% saturation proved to be the best precipitant for partially purified enzymes (cellulase and pectinase). Whilst 10mM glutaraldehyde concentration at 3h was found to be the best cross-linking time. Both enzymes were found to be stable for 5h at pH 5, 28 $^{o}$C and 50$^{o}$C. With respect to substrate specificity 2.0% and 2.5% were optimal for pectinase and cellulase activity respectively. African star apple peels which are usually discarded as waste can serve an avenue for the production of pectinase and cellulase enzyme used for commercial fruit clarification, textile processing.

## P153

# Beyond fluorescein: Use of fluorescent protein calibrants for direct and absolute quantification of protein production in synthetic biology

Eszter Csibra, Guy-Bart Stan
Imperial College London, London, United Kingdom

## Abstract

While inter-lab calibration standards are approaching mainstream usage in synthetic biology, such calibrations are not in fact sufficient for absolute protein quantification required for modelling synthetic circuits. Fluorescein-based calibration of plate reader and flow cytometry instruments allows the measurement of green fluorescent protein (GFP) in synthetic cells to graduate from arbitrary units to calibrated units, but retains important caveats. Fluorescein is only a good calibrant for green FPs, leaving other FPs uncalibrated, and only provides conversions to units of brightness, not to molecule numbers.

Ideal assay calibrants in molecular biology consist of the same molecule as the one to be measured – in this case, a purified preparation of the appropriate fluorescent protein. Here we show that by using purified FP calibrants, all protein species in a synthetic circuit can be quantified in absolute terms using no advanced instrumentation. We develop a SEVA (Standardised European Vector Architecture)-based expression vector that allows the high-level production of soluble protein and describe a straightforward 2-day protocol for the purification of micrograms of FP, followed by a calibration that relates fluorescence activity to protein mass.

We validate this protocol by calculating conversion factors for a panel of commonly-used FPs including superfolder GFP, mCherry, mScarlet-I and mTagBFP2 on multiple laboratory instruments, and use these calibrations to debug synthetic circuits. We also demonstrate that the suspected bias of the presence of mCherry on OD600 measurements is real, but in practice requires extreme overexpression (~100,000 proteins per cell) to have a meaningful impact on cell density estimates.

## P155

# Parallel phage resistance - virulence trade - offs during clinical phage therapy and in vitro

Meaghan Castledine[1], Daniel Padfield[1], Pawel Sierocinski[1], Jesica Soria Pascual[1], Adam Hughes[1], Lotta Mäkinen[1], Ville-Petri Friman[2], Jean-Paul Pirnay[3], Daniel De Vos[3], Angus Buckling[1]
[1]University of Exeter, Penryn, United Kingdom. [2]University of York, York, United Kingdom. [3]Queen Astrid Military Hospital, Brussels, Belgium

## Abstract

With rising antibiotic resistance, modern medicine needs new approaches for

tackling bacterial infections. Phage therapy uses the viruses of pathogenic bacteria to clear the infection. Unlike antibiotics, phage can evolve if bacteria become resistant to maintain or even increase their infectivity (coevolution). While laboratory studies can give insight into complex bacteria-phage interactions, whether they act as a true representation of phage therapy in patients is unknown. Here, we compared phage therapy in a patient to that of in vitro experiments. The patient had been admitted with a Pseudomonas aeruginosa infection and was successfully treated with a phage cocktail. Bacteria were isolated before and during phage therapy, allowing us to follow bacteria-phage coevolution in the patient while doing experiments on the same clones in vitro. In vivo and in vitro, bacteria rapidly evolved resistance with little or no evidence of bacteria - phage coevolution. Although resistance mechanisms differed, parallel resistance - virulence trade-offs were found in vivo and in vitro. Therefore, phage resistance could increase treatment success and our results indicate to what extent bacteria - phage evolutionary dynamics can be predicted from in vitro experiments.

## P156

# Phage display strategy against outbreaks of unknown pathogens

Marco Palma
Independent Researcher, Torrevieja, Spain

## Abstract

The experience with SARS-CoV-2 that spread rapidly throughout the world makes us realize we need protocols to act quickly against unknown pathogens. The immune system protects recovering patients from any pathogen by producing antibodies against their immunogenic epitopes. Therefore, the strategy presented here is based on convalescent blood samples and a phage display platform for antibody and peptide drug discovery. Peptide libraries are screened against purified convalescent antibodies to identify immunogenic epitopes of the pathogen. Furthermore, the B cells of the recovery patients are used to amplify variable domains of antibody heavy and light chains expressed during the infection. These domains are cloned in a phagemid and produce free phage particles expressing the antibody fragments on their surfaces to select binders to pathogen immunogenic epitopes. These findings are essential in the identification of the unknown pathogen and the design of therapeutic molecules. In conclusion, this report describes a phage display strategy to combat outbreaks of unknown pathogens such as SARS-CoV-2.

## P157

# Improving phage genome annotation to understand phage biology: the case of *Pseudomonas aeruginosa* LES prophages.

Enrique Gonzalez-Tortuero[1], Revathy Krishnamurthi[2], Ian Goodhead[1], Heather Allison[2], Chloe James[1]
[1]School of Science, Engineering and Environment, University of Salford, Salford, United Kingdom.
[2]Department of Clinical Infection, Microbiology and Immunology, Institute of Infection, Veterinary and Ecological Sciences (IVES), University of Liverpool, Liverpool, United Kingdom

## Abstract

*Pseudomonas aeruginosa* is an important opportunistic pathogen, causing nosocomial infections. The Liverpool Epidemic Strain (LES), a major cause of mortality and morbidity in cystic fibrosis patients, harbours five prophages associated with increased fitness and survival in models of infection. However, ~76.5% of the LES prophage genes are hypothetical proteins. Also, little is known about the LES prophage interactions with the lysogen and other prophages. In this study, we used the VIral Genome Annotation (VIGA) pipeline to re-annotate the LES prophage genomes and improve the prediction of gene function. The RefSeq viral proteins database was used for homology-based gene prediction and the Prokaryotic Virus Orthologous Genes (PVOGs) and Reference Viral DataBase (RVDB) were used for HMM-based protein function prediction. InterProScan 5.47-5.82 and Infernal 1.1.3 (with Rfam 14.3) were applied to enrich the gene function prediction for all genes and ncRNA elements, respectively. The number of putative coding sequences had increased by 1.17-1.43 times. Multiple genes related to DNA recombination and host cell lysis were identified in this reannotation. Also, we have identified new ncRNA elements in these prophages, such as tRNAs in prophages 2 and 5 and a putative regulatory ncRNA, misidentified as the plant viral Hammerhead-II ribozyme, in prophage 4. All this new information will be combined with data from future RNAseq experiments to map the expression profiles of each LES prophage under inducing and non-inducing conditions to characterise interactions between the prophages and their lysogen host.

**P158**

# Investigation of the Localisation of FtsZ in Pathogenic E. coli Upon Bacteriophage Addition in a Human Cell Model as a Biomarker for Antibacterial Agents.

Gurneet Dhanoa[1], David Roper[1,2], Antonia Sagona[1]
[1]School of Life Sciences, University of Warwick, Coventry, United Kingdom. [2]University of Columbia, New York, USA

## Abstract

Antimicrobial resistance is a growing problem worldwide and has created a need for novel antibacterial agents and strategies. *Escherichia coli* is one of the most common Gram-negative pathogens and is responsible for infection leading to neonatal meningitis and sepsis. The FtsZ protein is a bacterial tubulin homolog required for cell division in most species, including *E. coli*. Agents that block cell division have been shown to mis-localise FtsZ, including the bacteriophage λ encoded Kil peptide, resulting in defective cell division and a filamentous phenotype, and therefore FtsZ may be an attractive target for new antimicrobials.

In this project, we are interested in studying the localisation of FtsZ in pathogenic *E. coli* in the presence and absence of human cell cultures, in order to establish how and if this localisation changes upon infection. We are also interested in whether bacteriophages specifically attacking pathogenic *E. coli* have an effect on the localisation of FtsZ in a human cell environment and want to study the mechanism of this process. We have observed *E. coli* FtsZ localising to the cell midbody as a ring in both a K12 strain and in the K1 strain EV36 using confocal microscopy. These strains were used to infect human cerebral microvascular endothelial cells (hCMEC/D3) to create a meningitis model. We will present our results showing the effect of the Kil peptide and bacteriophages on this localisation within the model system, in an effort to validate FtsZ as a potential biomarker for antibacterial agents.

**P159**

# Multi-omics analysis of *Clostridium saccharoperbutylacetonicum* responses to product and feedstock inhibitor mediated stress

Benjamin J. Willson[1], Reyme Herman[1], Vicki Springthorpe[1], Jared Cartwright[1], Sandy Macdonald[1], David-Paul Minde[2], Swen Langer[1], Joseph Webb[3], Vivien Yeh[4], Alice Goode[4], Preben Krabben[5], Elizabeth R. Jenkinson[5], Tony Larson[1], David J. Kelly[3], Jeffrey Green[3], Boyan Bonev[4], Kathryn S. Lilley[2], Gavin H. Thomas[1]

[1]Department of Biology, University of York, York, United Kingdom. [2]Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom. [3]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, United Kingdom. [4]School of Life Sciences, University of Nottingham, Nottingham, United Kingdom. [5]formerly Green Biologics Ltd, Milton Park, Abingdon, United Kingdom

## Abstract

The effects of climate change and limited fossil fuel reserves are driving renewed interest in sustainable sources of energy such as biofuels. While ethanol is currently the most widely used biofuel, the use of butanol as a fuel has several advantages, especially if produced from sustainable lignocellulosic sources. However, while butanol has historically been produced industrially, using microbes from the genus *Clostridium*, the bioproduction of butanol is currently economically unfavourable. Thus, we are aiming to improve the industrially relevant bacterium *Clostridium saccharoperbutylacetonicum* as a bioproduction chassis by increasing its tolerance to the fermentation product *n*-butanol, as well as the inhibitory compounds furfural and hydroxymethylfurfural, which are typically found in lignocellulose-derived feedstocks. To identify potential gene candidates, we spiked *C. saccharoperbutylacetonicum* cultures grown in chemostatically controlled batch fermentations with butanol, furfural and hydroxymethylfurfural and carried out multi-omics analyses over a short time course. These include deep proteomics analyses which collectively provided direct evidence for 3281 predicted *C. saccharoperbutylacetonicum* proteins and comprehensive quantitative insights into their dynamics upon toxic challenge. This complex dataset has been interrogated using MORF, an in-house developed database for integration of multi-omics data. From this, we have identified multiple potential resistance candidates, including genes involved in stress response, metabolism and efflux, providing a strong basis for further developments.

## P160

# An optimised phage cocktail targeting pathogenic *Klebsiella* in an animal model.

Lucy Kelly, Antonia Sagona, Meera Unnikrishnan, Eleanor Jameson
University of Warwick, Coventry, United Kingdom

## Abstract

Antibiotic-resistant *Klebsiella* are increasingly becoming a threat to human health, causing diseases such as pneumonia, urinary tract infections and meningitis. Bacteriophage (phage) are viruses that infect and kill bacteria, offering a promising potential alternative to antibiotic treatment.

Here I present a cocktail of 7 *Klebsiella* phage to target multiple strains of *Klebsiella*. *In vitro* culture studies were used to first evaluate the efficacy of the phage cocktail against *Klebsiella* strains, using the virulence index as a quantitative measure of phage cocktail virulence. Both 96-well plate- and 10 mL MicrobeMeter-based methods were used to analyse the optimal dosing concentration for administering the phage cocktail.

An optimised dose of phage cocktail was applied to a *Galleria mellonella* animal model to determine how a phage cocktail treatment works *in vivo*. By studying the survival of *G. mellonella* infected with *Klebsiella* we can begin to understand how effective the phage cocktail is when applied to an animal with an innate immune system and a complex microbiome.

The phage cocktail greatly inhibited the growth of all of the strains of *Klebsiella* tested, with a virulence index ranging from 0.29-0.68, dependent on the *Klebsiella* strain. These results demonstrate the efficacy of the optimised phage cocktail, suggesting such a treatment could be used as an accompaniment, or alternative, to antibiotic treatment in *Klebsiella* infection.

## P161

# Isolation and characterization of bacteriophage Ib_pec2 against Shigatoxigenic *E.coli*

Sophia Inbaraj[1], Pallab Chaudhuri[2], Prasad Thomas[2], Angappan M[2], Manish Kumar[2], Abhishek Verma[2], Karuna Irungbam[2], Viswas KN[2], Ravi Kant Agrawal[2]
[1]ICAR-IVRI, Bareilly, India. [2]ICAR-Indian Veterinary Research Institute, Bareilly, India

## Abstract

The current study aimed to isolate and characterize bacteriophage against drug resistant, Shiga toxigenic *E.coli*, one of the zoonotic, food-borne organisms associated with ruminants, mainly cattle. STEC were isolated (n=35) from neonatal calves, dairy workers and the surrounding environment and their antimicrobial resistance pattern was studied. Out of the 35 isolates tested, 17 isolates were found to be multi-drug resistant to important antibiotics like as ampicillin, amoxicillin-clavulanate, ciprofloxacin, streptomycin and tetracycline. Bacteriophage namely Ib_pec2 was isolated against NM—18-040 isolate and its morphology, genetic and proteomic characterization was done. Morphological analysis by TEM revealed bacteriophage belonging to myoviridae family. The genetic characterization of g23 gene revealed that the bacteriophage belonged to Tequatrovirus of myoviridae family. SDS-PAGE analysis of structural proteins followed by LC-MS/MS analysis could able to identify five proteins identical to Tequatrovirus of myoviridae family. One step growth curve experiments revealed a latency period of 40 mins and a burst size of 893 PFU/ bacteria. Temperature and pH ranging from 40-50°C, pH 7-8, respectively were ideal for phage survival and multiplication. Phage was able to lyse 22 out of 35 STEC isolates. Thus, the study could able to characterize Ib_pec2 which could be used in control of STEC in the near future. STEC is a commensal organism in the gastrointestinal tract of ruminants but pathogenic in humans. Bacteriophages can be used as alternatives to antibiotics to control its growth in ruminants and prevent its further spillage in the environment.

## P162

# Benchmarking metagenomic phage identification tools

Siu Fung Ho[1], Andrew Millard[2], Willem van Schaik[1]
[1]University of Birmingham, Birmingham, United Kingdom. [2]University of Leicester, Leicester, United Kingdom

## Abstract

As the relevance of phages in shaping diversity in microbial ecosystems is becoming increasingly clear, the prediction of phage sequences in metagenomic datasets has become a topic of considerable interest, leading to the development of many novel bioinformatic tools. However, to our knowledge, there has not been a comprehensive comparison of these methods.

We benchmarked 10 state-of-the-art phage identification tools using three datasets. Artificial contigs generated from complete RefSeq genomes and a previously sequenced mock uneven community containing 4 phage strains were used to evaluate the precision, recall and F1-scores. In addition, a set of previously simulated viromes was used to assess diversity bias in each tool's output.

DeepVirFinder performed best across the first two datasets, with the highest F1-scores (0.98 and 0.61 respectively). Generally, machine learning- based tools performed better on the first dataset and both reference and machine learning based tool performed comparably on the second dataset. Most tools produced a viral genome set that had similar alpha and beta diversity patterns to the original population with the notable exception of Seeker, whose output differed from the diversity of the underlying data.

This study provides key metrics used to assess performance of phage detection tools, offers a framework for further comparison of additional viral discovery tools, and discusses optimal strategies for using these tools.

## P163

# Global diversity and potential functioning of prophages in plant pathogenic *Ralstonia solanacearum* bacterium

Samuel Greenrod, Martina Stoycheva, Ville-Petri Friman
University of York, York, United Kingdom

## Abstract

*Ralstonia solanacearum* is a destructive plant pathogenic bacterium which harbours a wide variety of virulence genes allowing it to infect over 200 plant species worldwide. Its virulence is also affected by the presence of integrated bacteriophages, termed prophages. While several such prophages have been identified, the global distribution and diversity of *R. solanacearum* prophages is unknown.

To study this, we first identified prophages present in a diverse collection of 192 assembled *R. solanacearum* genomes. Prophage diversity was explored by calculating prophage genetic distances and clustering with characterised prophages in a neighbour-joining tree. Prophage clusters were further verified by assessing gene content, GC content, and prophage length. Prophage identities were determined using the NCBI Virus database, and prophage-encoded virulence genes identified by analysing pangenome content.

343 intact prophages were identified, forming ten prophage clusters with distinct gene content, GC content, and length profiles. Five prophage clusters, containing 159 prophages, belonged to the *Inoviridae*, *Myoviridae*, and *Siphoviridae* phage families. The remaining 184 prophages were uncharacterised and may therefore represent novel prophages. Transcriptional regulators with potential virulence effects were identified in three prophage clusters, including one uncharacterised cluster. These prophage clusters were unequally distributed throughout the *R. solanacearum* population being host genotype specific.

This research demonstrates that *R. solanacearum* contains a high level of uncharacterised prophage diversity and highlights novel prophages that could contribute to pathogen virulence. Given their potential host-genotype-specific virulence effects, *R. solanacearum* prophages could be co-evolving with their hosts, and may contribute to global variation in *R. solanacearum* virulence.

**P164**

# Isolation and characterisation of bacteriophages for the biocontrol of *Klebsiella* spp.

Claire Elek, Teagan Brown, Evelien Adriaenssens
Quadram Institute Bioscience, Norwich, United Kingdom

## Abstract

*Klebsiella* spp. are common commensals of human mucosae, presenting a major risk factor for developing invasive disease and are therefore important opportunistic pathogens. Antibiotic resistance among *Klebsiella* spp. represent a major threat to human health, with many isolates now multidrug resistant. Bacteriophage therapy has seen a resurgence in recent years as an alternative or adjunctive to current antibiotic therapy.

The discovery of several novel bacteriophages (phages) has enabled the building of a phage bank against a range of clinical, food, and environmental *Klebsiella* host strains. Genomic classification and preliminary phage infection experiments have determined which of these phages to take forward for further characterisation. Phage-antibiotic interactions will then be investigated to determine whether multidrug resistance in *Klebsiella* spp. can be prevented or reversed.

Together, these data will determine which phages will be the most suitable candidates for phage therapy and a phage therapy cocktail will be developed as an alternative or adjunctive to antibiotics.

## P166

# Bacteriophages as biocontrol agents for plant diseases caused by Burkholderia bacteria

Maya Wright, Rebecca Weiser
Microbiomes, Microbes and Informatics Group, School of Biosciences, Cardiff University, Cardiff, United Kingdom

## Abstract

The genus *Burkholderia* is diverse and contains important pathogens of food crops such as rice and onions, and flowering plants such as orchids. Currently, there are few control measures or treatments for *Burkholderia* plant disease. We sought to investigate bacteriophages as a novel biocontrol approach. The aims of this Harry Smith Vacation Studentship were to (i) isolate bacteriophages active against the plant pathogen *B. gladioli* and, (ii) test these bacteriophages as a treatment for soft rot in mushrooms.

Twenty Burkholderia genomes, representing 17 different species, were screened bioinformatically to identify prophages. Strains containing intact prophages were assayed for spontaneous prophage induction against *B. gladioli* hosts. High titer stocks of isolated phages were evaluated for their ability to treat mushroom rot at different multiplicities of infection (MOI; 1, 0,1 and 0.01), using a *B. gladioli* mushroom rot assay optimised as part of the project.

Out of the seventeen *Burkholderia* strains predicted to carry intact prophages, ten were chosen for spontaneous prophage induction. Two novel bacteriophages were isolated from *B. ambifaria* and *B. pyrrocinia*, which had lytic activity against a range of *B. gladioli* strains. Phage application to the *B. gladioli* mushroom rot model did not improve the symptoms of infection, and at the higher MOIs (1 and 0.1) appeared to increase rotting.

Novel bacteriophages may be isolated from lysogenic *Burkholderia* that are active against other Burkholderia species. Whilst phage treatment of mushroom rot was not successful in our model, there is great potential for the use of phage biocontrol in agriculture.

## P167

# An interdisciplinary approach to reveal the dynamics of generalized transduction of antimicrobial resistance genes

Quentin Leclerc[1], Arya Gupta[2], Jodi Lindsay[3], Gwenan Knight[1]
[1]London School of Hygiene & Tropical Medicine, London, United Kingdom. [2]University of Kent, Canterbury, United Kingdom. [3]St George's University of London, London, United Kingdom

### Abstract

Background: Antimicrobial resistance (AMR) genes can spread between bacteria by "generalized transduction", where phages act as vectors to transfer them. However, our knowledge of the dynamics of transduction and how to best represent them is limited. We aimed to fill this gap through an interdisciplinary approach, generating microbiological data and using mathematical models to clarify the underlying transduction dynamics.

Methods: We co-cultured two library strains of Methicillin-resistant Staphylococcus aureus, each harbouring a resistance gene for a different antibiotic, with 80α generalized transducing phage. We recorded numbers of bacteria and phages at multiple time-points over 24h, using the presence of bacteria resistant to both antibiotics as evidence that transduction occurred. We developed and compared mathematical models of transduction based on how well they fit to the lab data.

Results: After a growth phase of 8h, bacteria and phage surprisingly coexisted at a stable equilibrium in our culture, the level of which was dependent on the initial concentration of phage. The rate of transducing phage generation was approximately 10-6 per lytic phage, sufficient to consistently generate double resistant bacteria, detectable after only 7h. Dynamics of transduction were best captured by a mathematical model in which the rate of phage infection slows as the bacteria population approaches carrying capacity.

Conclusions: The novel data and models generated provide valuable insights into the dynamics of transduction of AMR. This interdisciplinary framework could be extended to other bacterial species, and is the first step towards evaluating the impact of transduction on the overall public health consequences of AMR.

## P168

# Investigating the difference in phage susceptibility among MDR Salmonella Typhimurium phage types DT104 and DT104b

Manal Mohammed[1], Beata Orzechowska[2]
[1]University of Westminster, London, United Kingdom. [2]Public Health England, London, United Kingdom

## Abstract

The surge in mortality and morbidity rates caused by multi-drug resistant (MDR) bacteria, prompted a renewal of interest to bacteriophages (phages) as clinical therapeutics and natural biocontrol agents. Nevertheless, bacteria and phages are continually under the pressure of the evolutionary phage-host arms race for the survival, which is mediated by the co-evolving resistance mechanisms. In the phage typing scheme of *Salmonella* Typhimurium, the epidemiologically related definitive phage types; DT 104 and DT 104b display significantly different phage susceptibility profiles. This study aimed to characterise phage resistance mechanisms and genomic differences that may be responsible for the divergent phage reaction patterns in *S*. Typhimurium DT104 and DT104b using whole genome sequencing. The analysis of intact prophages, restriction-modification systems (RMS), plasmids and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) along with CRISPR-associated proteins, showed that the genomes of DT104 and DT104b harbour identical molecular determinants of phage resistance. Therefore, the findings may also propose the need for assessing phage-specific receptors on the bacterial cell surface, since, they are the initial point of host-phage interface. Building up the understanding of host-phage interactions will ultimately lead to the development of phage-based technologies enabling effective infection control.

**P169**

# Investigation of prophage carriage in the genus *Burkholderia* and characterisation of inducible phages from *Burkholderia vietnamiensis* strain G4

Rebecca Weiser[1], Zhong Ling Yap[1,2], Ashley Otter[1,3], Brian V. Jones[4], Jonathan Salvage[5], Julian Parkhill[6], Eshwar Mahenthiralingam[1]
[1]Cardiff University, Cardiff, United Kingdom. [2]University of Manitoba, Winnipeg, Canada. [3]Public Health England, Salisbury, United Kingdom. [4]University of Bath, Bath, United Kingdom. [5]University of Brighton, Brighton, United Kingdom. [6]University of Cambridge, Cambridge, United Kingdom

## Abstract

Members of the genus *Burkholderia* occupy diverse ecological niches and have environmental, industrial and medical importance. *Burkholderia* have large (6-9 Mb) multi-replicon genomes and 10% of this genetic material is estimated to be horizontally acquired. We aimed to investigate prophage carriage in *Burkholderia* genomes and isolate spontaneously inducible bacteriophages from *B. vietnamiensis* strain G4.

*Burkholderia* genomes were screened for prophages using PHASTER. For *B. vietamiensis*, the predicted intact prophages were compared using MASH and phylogenomic methods used to assess the distribution of phage clusters across *B. vietnamiensis* strains. Spontaneously induced phages were characterised to determine their morphology and host range, and genome sequenced to identify their corresponding genomic prophage regions.

Prophage carriage across 456 *Burkholderia* strains, spanning 43 species, was high; 716 intact prophages were discovered and polylysogeny was common. In *B. vietnamiensis* alone, 115 intact prophages were identified from 81 strains. Two inducible phages were isolated from *B. vietnamiensis* strain G4. Phage G4P1 was a Mu-like phage localised to chromosome 1, had lytic activity against strains of 5 *Burkholderia* species, and was highly similar (>93.5% tblastx identity) to prophages found in 17% of *B. vietnamiensis* strains. Phage G4P2 had a narrower host range and its genome sequence matched the entire length of the smallest plasmid of strain G4 (pBVIE05; 88 kb) indicating episomal carriage.

Prophages are numerous in *Burkholderia* genomes. There is huge potential for further investigation into functional implications of prophage carriage and its impact on genome evolution, in addition to the isolation of novel bacteriophages for biotechnological applications.

**P171**

# Do temperate bacteriophages work together as puppet masters of their bacterial hosts?

Revathy Krishnamurthi[1], Enrique González-Tortuero[2], Ian B Goodhead[2], Chloe E James[3], Heather E Allison[1]
[1]Department of Clinical Infection, Microbiology and Immunology, Institute of Infection, Veterinary and Ecological Sciences (IVES), University of Liverpool, Liverpool, United Kingdom. [2]School of Science, Engineering and Environment, University of Salford, Salford, United Kingdom. [3]Department of Clinical Infection, Microbiology and Immunology, Institute of Infection, Veterinary and Ecological Sciences (IVES), University of Liverpool, Salford, United Kingdom

## Abstract

*Pseudomonas aeruginosa* is one of the major causes of morbidity in cystic fibrosis (CF) patients. *The* Liverpool Epidemic Strain (LES) is associated with more severe disease and is transmissible between hosts. Comparative genomics revealed that the LES genome had obtained five new genomic islands and five novel prophages. Previous studies detected abundant free LES phages in CF patient sputum and isolated three active LES phages from LES cultures. These phages were found to confer fitness advantages to LES, but the specific mechanisms are not known. Our study aims at understanding the molecular mechanisms by which these prophages impact the biology of the *P. aeruginosa* host.

We used a well-characterised laboratory strain of *P. aeruginosa* (PAO1) that is susceptible to lysogenic infection by three LES phages. This enabled the construction of lysogen variants harbouring three LES prophages (specifically Φ2, Φ3 and Φ4) in all possible combinations, creating single, double and triple lysogens. Our preliminary comparison of growth characteristics revealed direct phage-specific effects and interactions between the prophages. Transcriptome studies of these lysogens under varying conditions will help to identify the specific interactions, which will be confirmed through mutant construction and phenotypic analyses.

IMPORTANCE: Our study shows that LES phages increase the fitness of their host through specific interactions. Further investigation in clinically relevant conditions could identify unique targets for tackling recalcitrant *P. aeruginosa* infections.

## P173

# What is the effect of bacteriophage treatment on the healthy gut microbiota?

Teagan Brown[1], Claire Elek[1], Rebecca Ansorge[1], Andrea Telatin[1], Elizabeth Kutter[2], Evelien Adriaenssens[1]
[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Evergreen State College, Olympia, USA

## Abstract

**Background**
The treatment of bacterial gastro-intestinal infections with antibiotics can have long-lasting effects on microbiome composition, with the potential for a dysbiotic state to be established. Bacteriophage preparations can be purchased "over-the-counter" in certain parts of the world to combat infection, but little is known about the effects these have on the native members of the gut microflora.

**Methods**
These experiments used the commercially available Intesti Bacteriophage cocktail manufactured by the Eliava Institute (Georgia). To model the gut, batch culture fermenters were seeded with faecal samples from three healthy individuals with one control and one phage-treated vessel per donor. To capture the diversity over time, the faecal slurry was sampled at phage addition (t=0), six-hours and 24-hours, DNA extracted and Illumina shotgun sequenced. The resulting sequences were analysed with various bioinformatic profiling tools including MetaPhlAn3 and Kraken2, to observe changes in the microflora.

**Results**
Regardless of the initial diversity, after six-hours there was a shift in the microbial community in all samples toward members of the *Enterobacteriaceae* family, driven by the model system conditions. At the bacterial species level, limited differences between phage-treated and control samples were observed. Some of the phages from the cocktail increased and decreased in abundance over the time course in a donor-dependent manner, suggesting productive infection.

**Conclusion**
The phage cocktail had minimal effects on the healthy gut microbiota indicating that it can be used as a treatment with few side effects on the microbiome. The donor-dependent phage bloom could indicate strain-level effects that warrant further investigation.

## P174

# Expanding viromes associated with *Brevundimonas* and *Serratia*

Ines Friedrich[1], Hannes Neubauer[1], Anna Klassen[1], Bernhard Bodenberger[1], Faina Tskhay[1], Sara Hartmann[1], Dominik Schneider[1], Robert Hertel[1,2], Rolf Daniel[1]
[1]Georg August University of Göttingen, Göttingen, Germany. [2]BTU Cottbus-Senftenberg, Senftenberg, Germany

## Abstract

The predominant use of dsDNA as genomic material of diverse bacterial viruses (phages) is indicated by many studies. Two new bacterial host systems, *Brevundimonas* and *Serratia*, were isolated, characterized and used as viral host-systems for isolation- and metagenomic-based phage investigation, to test this observation. Both bacterial strains were used for phage enrichment with water samples derived from the primary treatment step of a local sewage plant (Göttingen, Germany). We specifically targeted phages containing dsDNA, ssDNA, dsRNA or ssRNA as genetic material. In total, eight *Brevundimonas*-associated dsDNA phages ranging from 62 to 356 kb and nine *Serratia*-associated dsDNA phages, ranging from 39 to 278 kb were isolated. All of them use dsDNA as genomic material, and no isolates were obtained with a distinct genome type. Metagenomic data, however, could be obtained from all types of prepared nucleic acids. By combining genome data from the obtained isolates with the dsDNA metagenomic sequence pool, we showed that even stringent isolation covers in the best case only 70 to 90% of potential phages infecting a host present in a sample. To conclude, we could show that dsDNA phages are the easiest to obtain and represent presumably the predominant phage group. We exposed the need to expand our experimental procedures to obtain isolates with a different genome type. Furthermore, we show that the accessibility of a sample's phage diversity depends on the host, the sampling season and the combination of isolation- and metagenome-based approaches employed for its investigation.

## P175

# An investigation into the environmental isolation of bacteriophages effective against pandemic multidrug resistant *Escherichia coli* ST131.

Jessica Forsyth, Ben Raymond, Stineke Van Houte, Bridget Watson, Matthew Chisnall
University of Exeter, Cornwall, United Kingdom

## Abstract

The emergence and rapid dissemination of multidrug resistant (MDR) pathogens presents a huge and growing global concern. *Escherichia coli* (E. coli) sequence type (ST) 131, has been recognised as a major E. coli lineage responsible for the spread of MDR, and is a common cause of life-threatening infections in clinical settings. There is increasing interest in the use of bacteriophages in the treatment of MDR infections. The use of bacteriophages with some breadth of host range can be desirable so that licensed products are able to target a range of clinical infections. This study set out to investigate whether phages infective against ST131 could be found in sewage environments and to identify factors that would increase the chances of isolating broader host range phage. Phages infective against ST131 were confirmed to be present in samples obtained from four different classes of sewage environment: agricultural, clinical, community wastewater and activated sludge. Using a unique method of quantifying host range, this study also provides evidence that the type of sewage environment sampled from and the host strain used for isolation are both significant determinants of the host range of phages isolated. With the results of this study, we aim to contribute to the development of a systematic sampling framework that will improve the ability to obtain phages with the most promising therapeutic potential from natural and artificial environments.

**P179**

# Low risk term fetal membranes do not display a distinct bacterial load, independent of delivery method.

Rochelle Hockney[1], Caroline Orr[1], Gillian Taylor[1], Inge Christaens[2], Stephen Cummings[1], Andrew Nelson[3]
[1]Teesside University, Middlesbrough, United Kingdom. [2]Newcastle University, Newcastle, United Kingdom. [3]Northumbria University, Newcastle, United Kingdom

## Abstract

Bacterial colonisation of the fetal membranes *in utero* is questioned in healthy pregnancies, with key debates as to whether the fetal membranes have a specific bacterial signature which is influenced by mode of delivery. This research aims to determine if bacterial loads can be detected on low risk term fetal membranes and if the bacterial load and profile differs based on mode of delivery. This research employed a retrospective cohort study of fetal membrane rolls collected from patients with low risk term elective caesarean section (ELCS, n=44) or term spontaneous vaginal delivery (n=22). Bacterial profiles were investigated using 16S rRNA Illumina sequencing. Bacterial loads were measured by 16S rRNA BactQuant absolute qPCR. The majority of low risk term fetal membranes did not display a detectable bacterial signal using the methods employed (77%, 51/66), with a bacterial signal detected in 23% of ELCS (10/44) and vaginal deliveries (5/22). There was no significant difference in copy number between delivery types (p=0.977). The most abundant genera detected in low risk term fetal membranes was *Escherichia/Shigella. Lactobacillus iners* was significant greater in vaginal delivery (p=<0.001, FDR=<0.001). Alpha and beta diversity did not differ by mode of delivery (p=0.093, p=0.173). Decontam detected one contaminating ASV, belonging to *Cetobacterium* (p=0.030). Findings suggest that there is no distinct bacterial load in low risk term fetal membranes, with minimal bacterial contribution to the fetal membranes during delivery. Mode of delivery does not impact the bacterial load or diversity on low risk term fetal membranes.

## P180

# Bacteriological and Physicochemical Assessment of Sachet and Bottled Drinking Water Sold in Makurdi Metropolis, Benue State, Nigeria

Lubem Agbendeh, Innocent Ogbonna
University of Agriculture, Makurdi, Makurdi, Nigeria

## Abstract

Background: Packaged drinking water popularly known as "sachet and bottled water" serves a large percentage in increasing access to clean drinking water in Nigeria. But little attention is given to investigate the bacteriological assessment of these water which may be harmful for human consumption.

Objective: This study was to assess the bacteriological and physicochemical characteristics of sachet and bottled water sold in Makurdi metropolis.

Methodology: A cross sectional study was carried out with a total of one hundred and sixty-five samples collected. These comprised of triplicates of fifty sachet water and five bottled water brands purchased using simple random sampling from street vendors within Makurdi metropolis and analyzed using standard methods and results were compared with the recommended guidelines for water quality.

Results: The presence of Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus sp., Klebsiella sp., Salmonella sp. and Shigella sp. were detected in the water samples with a total bacteria count (MPN/100ml) ranging from 0 – 1100 MPN/100ml. Escherichia coli had the highest incidence of 80% followed by Staphylococcus aureus 66%, Salmonella species 48%, Pseudomonas aeruginosa 46%, Streptococcus sp 36%, Shigella species 36%, Klebsiella species 22%.The pH, conductivity, total dissolved solids, dissolved oxygen and sodium chloride levels ranged from 6.0 to 7.6, 8.4 to 188.4µS/cm, 4.2 to 94.2mg/l, 0.08 to 0.16mg/l and 0 to 0.4 respectively.

Conclusion: There is urgent need to intensify the monitoring of activities of this rapidly expanding industry and enforcing strict hygienic measures with a view to raising standards to improve packaged water quality.

**P182**

# Incidence of Bacterial Infection among Tuberculosis Suspects Attending Infectious Diseases Hospitals A. S.;1 Idris, A. M2 and Muktar, M. D.;11Department of Medical Microbiology and Parasitology, Faculty of Clinical Science, Collage of Health Science, Bayero University, Kano.*Corresponding Author: Abdurrahaman Mangari, shuaibuabdurrahaman@gmail,com, +234 7086575180.

Abdurrahaman Mangari[1], Mukhtar Dauda[2], Abdulrazak Idris[3]
[1]Bayer University, kano., Kano, Nigeria. [2]Bayer University kano, Kano, Nigeria. [3]Aminu Kano Teaching Hospital, Kano, Nigeria

## Abstract

Abstract

TB co-infection with other bacterial pathogens is one of the major health problems especially in TB endemic region. Bacterial pathogens that localized in the lower respiratory tract are could sometimes preclude MBT or manifest as pulmonary symptoms that could mislead clinicians. Therefore this study was planned to examine suspected TB patients in Kano, Nigeria for other pathogenic bacteria with view to establishing frequency of occurrence towards facilitating better outcome of therapeutic cover. A total of 170 non duplicated sputum samples were collected from patients with suspected pulmonary TB. All the samples were processed according to standard bacteriological procedures including macroscopic examination, culture and microscopic examination using both AFB staining and Gram staining methods. Isolated organisms were subjected to appropriate biochemical testes. High incidences were observed among age group 10 – 29 and 30 – 49 years with male and urban people been the predominant patients. Overall 20.6% of samples were AFB positive indicated TB co-infection while 62.4% were positive for other bacterial growth. Nalidixic acid and Nitrofurantoin found to have highest resistant among Gram negative organisms isolated following by Levofloxacin, Ceftriaxone and Ceftazidime while the Ofloxacin and Ciprofloxacin have the least resistant. Among Gram positive Ceftazidime shown to has the highest resistant and Ofloxacin found to has the least resistant. The study indicated predominance of S. pneumoniae (47.2%) and Staphylococcus species (32.1%) with an evidence of 15.1% and 2.8% co-infection wit TB.

## P183

# Evolutionary histories and antimicrobial resistance in *Shigella flexneri* and *Shigella sonnei* in Southeast Asia

Hao Chung The[1], Ladaporn Bodhidatta[2], Duy Thanh Pham[1,3], Carl J. Mason[2], Tuyen Ha Thanh[1], Phat Voong Vinh[1], Paul Turner[4,3], Sopheak Hem[5], David A.B. Dance[6,3,7], Paul N. Newton[6,3,7], Rattanaphone Phetsouvanh[6,3], Viengmon Davong[6], Guy E. Thwaites[1,3], Nicholas R. Thomson[8,7], Stephen Baker[9], Maia A. Rabaa[1,3]

[1]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam. [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. [3]Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, United Kingdom. [4]Cambodia-Oxford Medical Research Unit, Siem Reap, Cambodia. [5]Institut Pasteur du Cambodge, Phnom Penh, Cambodia. [6]Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Vientiane, Lao, People's Democratic Republic. [7]London School of Hygiene and Tropical Medicine, London, United Kingdom. [8]The Wellcome Trust Sanger Institute, Hinxton, United Kingdom. [9]The Department of Medicine, University of Cambridge, Cambridge, United Kingdom

## Abstract

Conventional disease surveillance for shigellosis in developing country settings relies on serotyping and low-resolution molecular typing, which fails to contextualise the evolutionary history of the genus. Here, we interrogated a collection of 1,804 *Shigella* whole genome sequences from organisms isolated in four continental Southeast Asian countries (Thailand, Vietnam, Laos, and Cambodia) over three decades to characterise the evolution of both *S. flexneri* and *S. sonnei*. We show that *S. sonnei* and each major *S. flexneri* serotype are comprised of genetically diverse populations, the majority of which were likely introduced into Southeast Asia in the 1970s-1990s. Intranational and regional dissemination allowed widespread propagation of both species across the region. Our data indicate that the epidemiology of *S. sonnei* and the major *S. flexneri* serotypes were characterised by frequent clonal replacement events, coinciding with changing susceptibility patterns against contemporaneous antimicrobials. We conclude that adaptation to antimicrobial pressure was pivotal to the recent evolutionary trajectory of *Shigella* in Southeast Asia.

## P184

# Novel methicillin-resistant *Staphylococcus aureus* sequence types isolated in a nursing home in Poland – pathogenicity and antibiotic resistance

Martyna Kasela, Agnieszka Grzegorczyk, Anna Malm
Medical University of Lublin, Lublin, Poland

## Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains an important etiological factor causing infections in elderly residing in nursing home (NH). Moreover, high pathogenicity of MRSA correlates with increased morbidity and mortality among infected individuals. We collected 21 MRSA strains from the residents and personnel in NH in Poland during 2018 year. All MRSA were screened for the presence of genes encoding enterotoxins A–E (*sea–see*), leucocidin Panton-Valentine (*lukS/F-PV*), exfoliative toxins (*eta*, *etb*), toxic-shock syndrome toxin (*tst*), adhesins (*bbp*, *can*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*) and *icaADBC* operon. Antibiotic resistance profile was determined using the Vitek 2 Compact and disc-diffusion method. The sequence type (ST) of MRSA was determined using multi-locus sequence typing (MLST) and staphylococcal cassette chromosome *mec* (SCC*mec*) typing. A much as 66.7% of MRSA were resistant only to β-lactam antibiotics, while 9.5% were classified as multi-drug resistant. All MRSA were negative for *sec*, *etb*, *eta* and *lukS/F-PV* genes. The most prevalent toxin gene was *sed* (52.4%). Adhesin genes present in all investigated MRSA were *eno* and *fnbA*. Typable MRSA strain were characterized by IV, V or VI SCC*mec* type. The MLST identified four STs among two Clonal Complexes (CC). We have identified three novel STs: ST6295 (CC8), ST6293 (CC8) and ST6294. The sequences have been deposited in MLST database. Longitudinal studies concerning MRSA colonization in NHs enable to assess actual MRSA distribution. The emergence of novel STs of unique pathogenicity and the presence of hospital-associated ST22-IV clone (EMRSA-15) create challenges for controlling the spread of MRSA in NHs.

## P185

# Changes in the upper respiratory tract microbiota composition during chemotherapy in group of patients with lung cancer

Edyta Ciebień[1], Urszula Kosikowska[1], Katarzyna Kurek[2], Barbara Mackiewicz[2], Dorota Pietras-Ożga[3], Anna Malm[4]
[1]Department of Pharmaceutical Microbiology, Medical University of Lublin, Chodzki Str. 1, Lublin, Poland. [2]Department of Pneumonology, Oncology and Allergology, Independent Public Clinical Hospital No. 4 in Lublin, Jaczewskiego Str. 8, Lublin, Poland. [3]Department of Epizootiology and Clinic of Infectious Diseases, University of Life Sciences in Lublin, Gleboka Str. 30, Lublin, Poland. [4]Department of Pharmaceutical Microbiology, Medical University of Lublin, Chodzki Str. 1,20-093 Lublin, Lublin, Poland

## Abstract

### Introduction

The lung cancer is among the most common types of carcinomas. Decrease health condition and immunity as well as a side effect of therapy may affect the increased colonization with opportunistic microorganisms that may endanger the patient's health or life.

### Methods

The study included 26 patients with lung cancer (LC-group) as study group and 30 healthy adults (HA-group) as control. Microorganisms were isolated from nasopharyngeal (NPX) and throat (TH) swabs. In the LC-group diagnostic material was taken before and after the 2nd cycle of chemotherapy. The materials were inoculated on microbiological media (blood and MacConkey agar). Isolates were identified using mass spectrometry (MALDI-TOF MS) technique. The effect of chemotherapy on the nasopharynx and throat colonization by Gram-negative rods was evaluated.

### Results

In the LC-group before chemotherapy, none of the patients was colonized with Gram-negative rods in NPX, while 4/26 (15.4%) ones were colonized in TH. During the chemotherapy this type of bacteria were selected in 2/26 (7.7%) patients in NPX and in 6/26 (23.1%) patients in TH. In HA-group 9/30 (30.0%) adults were colonized with Gram-negative rods in NPX and 9/30 (30.0%) ones in TH. The differences before and after chemotherapy were not statistically significant in NPX colonization ($p$ =0.1492) and in TH colonization ($p$=0.4816).

### Conclusions

Results shows that short chemotherapy does not cause a large impact on the number of Gram-negative rods colonizing the nasopharynx and throat of patients with lung cancer. However, to some extent, it can change the composition of the upper respiratory tract microbiota.

## P186

# Iron-regulated surface determinant B (IsdB) mediates *Staphylococcus aureus* interaction with the human hemostatic system by binding to von Willebrand factor (vWF)

Mariangela Jessica Alfeo[1], Giulia Barbieri[2], Pietro Speziale[1], Giampiero Pietrocola[1]
[1]Department of Molecular Medicine, Unit of Biochemistry, University of Pavia, Pavia, Italy. [2]Department of Biology and Biotechnology, University of Pavia, Pavia, Italy

## Abstract

*Staphylococcus aureus* is the causative agent of bloodstream infections causing high morbidity and mortality. Upon entry to the cardiovascular system, the pathogen faces the shear stress of flowing blood adhering to the endothelium through von Willebrand factor (vWF). Human vWF is a multimeric plasma glycoprotein stored in the Weibel-Palade bodies of vascular endothelial cells and α-granules of platelets. When released, the protein tends to unfold due to the shear forces, exposing cryptic binding sites and attaching to endothelial cells and platelets. *S. aureus* is known to interact with vWF via different proteins, such as SpA, vWFbp, and ClfA. Herein, *S. aureus* iron-regulated surface determinant B (IsdB) was identified as a new vWF receptor based on mass spectrometry data. Analysis of protein-ligand binding indicates a high-affinity interaction in the nanomolar range.

A1 domain of vWF was identified as the binding domain within vWF for IsdB. Analysis of the adhesion of *S. aureus* WT and mutant Δ*isdB* to immobilized vWF or A1 domain alone revealed that IsdB is a key vWF receptor in iron-starvation conditions, even though other surface proteins are involved in the binding. Interestingly, a significant increase in staphylococcal adherence to HUVEC monolayers was registered when the cells were treated with the calcium ionophore A23187 to promote vWF exposure. These findings suggest that, by mediating the binding between *S. aureus* and vWF, IsdB may contribute significantly to the pathogenesis of the endovascular staphylococcal disease.

## P187

# Palmatine and berberine chloride synergistically Inhibit NanH sialidase of *Tannerella forsythia*

Galleh P.R[1,2], Lambert D.W[1], Stafford G.P[1]
[1]School of Clinical Dentistry, University of, Sheffield, United Kingdom. [2]Nasarawa State University, Keffi, Nigeria

## Abstract

The periodontal pathogen *Tannerella forsythia* is associated with severe periodontitis, and expresses NanH sialidases that cleave sialic acids by hydrolyzing the glycosidic bonds to underlying sugars. Palmatine and berberine chloride are plant-derived alkaloids with pharmacological effects, including anti-inflammatory and anti-bacterial properties. Recombinant NanH sialidase was purified using HisTag affinity chromatography while sialidase activity was determined using 4-methylumbelliferyl *N*-acetyl-α-D-neuraminic acid (MUNANA) as a substrate. The individual and synergistic effects of palmatine and berberine chloride on NanH sialidase inhibition was determined as well as their antimicrobial effects. The IC50 values of palmatine and berberine chloride were found to be 0.143 and 0.474 mM respectively. A significant synergistic effect was observed when a 0.20 mM:0.50 mM Palmatine:Berberine chloride mixture was used, inhibiting NanH sialidase by almost 100%, as compared to 0.2 mM palmatine and 0.5 mM berberine chloride invidually, which inhibit sialidase activity by 60.33 and 55.94%, respectively. Additionally, an antimicrobial viability assay was conducted and, 0.5 mM palmatine and 0.45 mM berberine showed a significant antimicrobial activity against *Tannerella forsythia*. Lastly, to examine potential toxicity to host cells, the cytotoxic effects of palmatine (0.15 mM) on H357 oral squamous carcinoma cells was investigated using a trypan blue assay and palmatine was found not to be toxic. In summary, a combination of palmatine and berberine display significant synergistic inhibitory effects on NanH with minimal cytotoxic effects as well as potential antimicrobial effects on the oral pathogen *T. forsythia*. Suggesting that these compounds may have potential for future development.

Keywords: *Tannerella forsythia*, NanH sialidase, Palmatine, Berberine chloride, Synergistic effects

## P188

# Development of an infection responsive coating to control encrustation of urinary catheters.

Anthony J. Slate[1], Mina Kerio[2], Diana R. Alves[2], Scarlet Milo[3], Bhavik A. Patel[2], Jonathan Nzakizwanayo[1], Brian V. Jones[1]
[1]Dept of Biology and Biochemistry, University of Bath., Bath, United Kingdom. [2]School of Pharmacy and Biomolecular Sciences, University of Brighton., Brighton, United Kingdom. [3]Dept of Chemistry, University of Bath., Bath, United Kingdom

## Abstract

Indwelling urethral catheters are used extensively for long-term bladder management. However, they are associated with high infection rates; catheter associated urinary tract infections (CAUTIs) are the most prevalent source of hospital acquired infection. A leading cause of CAUTIs is *Proteus mirabilis* due to key virulence factors, one example is extensive urease production. Colonisation results in crystalline biofilm formation and subsequent catheter occlusion which obstructs urine flow and can lead to serious medical complications. To address this increasing medical concern, we developed an infection-responsive, dual-layer coating strategy to control urease activity in the bladder. The coating consisted of a pH sensitive upper coating of Poly(methyl methacrylate-co-methacrylic acid) (Eudragit S100®) and an under layer of Poly(vinyl alcohol) with an embedded urease inhibitor, Lithostat (Acetohydroxamic acid). When the pH level of the urine was elevated above 7.0 (due to urease production by *P. mirabilis*), the upper layer dissolved which resulted in Lithostat release. An *In vitro* model of the catheterised urinary tract was used to evaluate the coating efficacy. These experiments demonstrated a significant increase in blockage time due to a reduction in encrustation when 5 mg $mL^{-1}$ Lithostat was incorporated into the catheter coating. The release of Lithostat from the coating was quantified using a colorimetric urease assay, whilst the effect of Lithostat on catheter encrustation was assessed using flame photometry. The described infection-responsive catheter-coating constitutes a promising strategy for localised delivery of Lithostat in direct response to *P. mirabilis* colonisation, resulting in a reduction in biofilm formation and delayed catheter blockage.

## P189

# Genomic Epidemiology of *Shigella* in South Africa

George Stenhouse[1], Rebecca Bengtsson[1], Karen Keddy[2], Roy Roy Chaudhuri[3], Juno Thomas[4], Miren Iturriza-Gomara[1], Neil Hall[5], Anthony Smith[4], Kate Baker[1]
[1]University of Liverpool, Liverpool, United Kingdom. [2]Tuberculosis Platform, South African Medical Research Council, Pretoria, South Africa. [3]University of Sheffield, Sheffeild, United Kingdom. [4]Centre for Enteric Diseases, National Institute for Communicable Diseases, Johannesburg, South Africa. [5]Earlham Institute, Norwich, United Kingdom

## Abstract

*Shigella* is a leading cause mortality and morbidity from moderate to severe diarrhoea globally, predominantly affecting children under the age of five years living in low- and middle-income nations. Widespread multidrug resistance (MDR) and emerging resistance to the remaining treatments threatens to reverse hard won reductions in shigellosis mortality. While whole genome sequence analysis (WGSA) has been effectively used to further understanding of *Shigella* epidemiology, antimicrobial resistance (AMR) determinants and spread, ultimately aiding effective public healthcare policy, it has been under-utilised in sub-Saharan Africa. This ongoing study uses WGSA of a large, representative sub-sample of isolates from South Africa, collected as part of public healthcare surveillance from 2011 to 2016, and focuses on *Shigella flexneri* 2A and *Shigella sonnei*, as these endemic serotypes cause approximately half of all shigellosis cases in South Africa. We observed a significant disparity in the average age of infection between men and women, suggestive of secondary infection in the home. Preliminary results show that the isolates are highly related, with 98.5% of *S. sonnei* isolates belonging to clade 3.7 and 91.7% of *S. flexneri* 2A isolates forming two subclusters of Phylogroup 3, and that transmission between provinces is common. Through AMR genotyping, supported by incomplete phenotype data, we also identified widespread MDR for both serotypes, including two *S. sonnei* isolates resistant to extended spectrum Beta lactams. We plan to further examine the prevalence and transmission of strains and AMR determinants in the country and the role of secondary transmission through phylogenetic transmission modelling.

## P190

# Investigation of the impact of food preservatives on avian pathogenic *Escherichia coli* (APEC) and their role in driving zoonotic disease

Ghaith Fallata[1,2], Donal Wall[1]
[1]University of Glasgow, Glasgow, United Kingdom. [2]king saud bin abdulaziz university for health sciences, Riyadh, Saudi Arabia

## Abstract

The excessive use of antibiotics in agriculture is routinely described as a major contributor to bacterial antimicrobial resistance. Globally, antibiotics are also widely used as growth supplements in livestock. This has led to concerns regarding use of human-use antibiotics in food and food-producing animals. In more recent times organic acids such as propionic acid (PA) and formic acid (FA) have been used as alternative antimicrobials or preservatives in place of antibiotics.

PA is a short chain fatty acid naturally abundant in the human and animal intestine as a breakdown product of non-digestible carbohydrates. In the human intestine it plays important roles in regulating the immune response in the human body. Recently, a study has shown that exposure of a Crohn's Disease associated bacterial pathotype, adherent-invasive *Escherichia coli* (AIEC),to PA significantly altered its phenotype resulting in increased adhesion and invasion of epithelial cells and increased persistence through biofilm-formation.

AIEC are both evolutionarily and phylogenetically related to avian pathogenic *Escherichia coli* (APEC). PA and FA use is widespread in chickens a known source of zoonotic disease. Our results indicate that virulence of some APEC strains is increased by exposure to alternative antimicrobials such as PA and FA. This included increased adhesion of APEC strain *E. coli* 601 to human intestinal epithelial cells after exposure to FA which could be a potential risk of zoonotic disease. Further investigation of APEC strains is currently underway in a fermentation model of the poultry gut. This approach will improve our understanding of how commonly used.

## P191

# Genomic characterisation of *Streptococcus pyogenes emm*5.23, a recently emerged clade causing invasive disease in Scotland

Davide Pagnossin[1,2], Andrew Smith[3,4], William Weir[2], Chiara Crestani[1], Diane Lindsay[3], Roisin Ure[3], Katarina Oravcova[1]
[1]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, United Kingdom. [2]School of Veterinary Medicine, University of Glasgow, Glasgow, United Kingdom. [3]Bacterial Respiratory Infection Service, Scottish Microbiology Reference Laboratory, Glasgow Royal Infirmary, Glasgow, United Kingdom. [4]College of Medical, Veterinary & Life Sciences, Glasgow Dental Hospital & School, University of Glasgow, Glasgow, United Kingdom

## Abstract

*Streptococcus pyogenes* is a human pathogen that can cause a wide range of infections including invasive disease. Strains of this bacterium can be classified into *emm* types, some of which are considered particularly virulent. In this work, we genomically characterised a recently emerged population, the invasive *emm* type 5.23, that has become numerically relevant in Scotland since 2018, representing 4.71% and 9.82% of all invasive disease cases in 2018 and 2019, respectively.

We sequenced the genomes of all *emm*5.23 strains isolated in Scotland (n=51) until January 2020. We then assessed their virulence gene and antimicrobial resistance (AMR) determinants. To investigate the presence of mobile genetic elements (MGEs), we performed MinION sequencing and generated three closed and annotated *emm*5.23 reference genomes. All *emm*5.23 strains were mapped to the newly generated reference and the population structure investigated by building a maximum-likelihood phylogeny.

Our results suggest that the virulence gene profile of the Scottish *emm*5.23 isolates does not differ markedly from that of the wider *S. pyogenes* population. All the isolates carried AMR determinants predisposing them to express a resistant phenotype towards lincosamides, macrolides, streptogramin and tetracyclines. All isolates carried five MGEs consisting of four prophages and one composite element, the latter containing the AMR-conferring gen, *tet*M. The population structure indicated divergent evolution into two groups, one of which comprised the majority (84%) of the strains. The latter group shared three unique mutations that might account for the apparent higher infectivity of this *emm* type, and which will be subject to further analysis.

**P195**

# The anti-biofilm activity of metal complexes incorporating 1,10-phenanthroline ligands in clinical isolates of *Pseudomonas aeruginosa* from Irish Cystic Fibrosis patients

Megan O'Shaughnessy[1,2], Orla Howe[1,2], Michael Devereux[1]
[1]Technological University Dublin- City Campus, Centre for Biomimetics and Therapeutics, Dublin, Ireland.
[2]Technological University Dublin- City Campus, School of Biological and Health Sciences, Dublin, Ireland

## Abstract

*Pseudomonas aeruginosa* is an opportunistic pathogen and the leading cause of morbidity and mortality in cystic fibrosis (CF) patients in Ireland. Chronic *P. aeruginosa* infections are difficult to treat due to several antibiotic resistance mechanisms and the organism's propensity to form biofilms. In the current 'resistance era', there is a pressing need to develop alternative drugs to combat infections such as *P. aeruginosa*, that work alone or in combination with current antibiotics to ameliorate their effects. 1,10-phenanthroline (phen) is a heterocyclic organic compound which when coordinated to metals (metal-phen complexes) has been previously shown to have antimicrobial activity across a spectrum of pathogenic organisms. Complexes containing 1,10-phenanthroline-based ligands offer significant potential as novel antimicrobial agents to tackle resistant pathogens, with mechanisms of action different to those of common drugs in clinical use.

The effects of the metal-phen complexes (where the metal = $Cu^{2+}$, $Mn^{2+}$, and $Ag^{+}$) and gentamicin against *P. aeruginosa* isolated from CF patients, alone and in combination, were investigated on planktonic growth, biofilm formation and mature biofilm. To further extrapolate a potential mode of action; exopolysaccharide, extracellular DNA (eDNA), pyocyanin and pyoverdine components of the extracellular matrix of *P. aeruginosa* biofilms were also assessed. All three metal-phen complexes showed comparable and often superior activity to gentamicin in the CF strains, with respect to both biofilm formation and established biofilms. Combination studies presented synergistic activity between all three complexes and gentamicin, particularly for the post-treatment of established mature biofilms, and was supported by the reduction of the individual biofilm components examined.

## P196

# The evolution of Pseudomonas aeruginosa during short-term acute respiratory infections

Julio Diaz Caballero
University of Oxford, Oxford, United Kingdom

## Abstract

Antimicrobial agents have facilitated the treatment of recalcitrant bacterial infections, thus the rise and spread of bacteria resistant to these antibiotics pose a major threat to health care. *Pseudomonas aeruginosa*, one of the most common opportunistic pathogens, is associated with increased rates of morbidity and mortality in nosocomial facilities. Moreover, *P. aeruginosa*'s ability to rapidly become resistant to the antibiotic therapy administered during infection is a pressing problem worldwide. Through the fine-scale characterization of within-host *P. aeruginosa* isolates collected from patients across hospitals in Europe, we aim to understand the evolutionary drivers of antimicrobial resistance in this pathogen.

*P. aeruginosa* isolates were collected from patients in intensive care units across Europe over a longitudinal sampling of short-term acute respiratory infections. To characterise the diversity of infecting *P. aeruginosa*populations in-depth, twelve isolates per sample were acquired from each patient. High-throughput phenotyping of growth rate and antibiotic resistance profile were combined with whole-genome characterisation using short-read sequencing technologies for each collected isolate.

We observed phenotypic and genotypic diversity between samples spanning few days, and even within the same sample. Genetic diversity was observed at multiple resolution levels. Namely, we identified not only patients co-infected by *P. aeruginosa* with distinct multi-locus sequence typing (MLST) but also patients infected by *P. aeruginosa* with identical MLST, which diversified through single nucleotide polymorphisms and insertions or deletions throughout the course of the infection. This data set provides an unprecedented level of insight into the population biology of antibiotic resistance in this pathogen.

## P197

# Immuno-proteomics of sera from gonorrhoea patients identified potential vaccine candidates

Aiste Dijokaite, Victoria Maria Humbert, Paul Skipp, Myron Christodoulides
University of Southampton, Southampton, United Kingdom

## Abstract

Gonorrhea is a sexually transmitted disease caused by *Neisseria gonorrhoeae* and is one of the leading reportable STDs in adults, with ~87 million cases annually and globally. Gonorrhoea remains a major global public health concern not only because of rising incidence each year, but also because of rising antimicrobial resistance. There is an urgent need for long-term solutions to prevent gonorrhoea such as vaccines, but none currently exist and research is focused on identifying potential antigens for inclusion in new vaccines. In our study, we used an immuno-proteomics approach to try and identify potential vaccine candidates.

A heterologous *N. gonorrhoeae* strain P9-17 was grown under iron-limiting conditions and whole cell lysates prepared. These were separated by isoelectric focusing (pH 3-10 range) and fractions were separated by SDS-PAGE. Western blots were prepared and reacted with sera from 20 patients with uncomplicated gonorrhoea and with sera from 5 controls with no history of gonorrhoea. Immuno-reactive bands were excised from the corresponding gels and subjected to mass spectrometry, which provided a profile of gonococcal proteins. After comparing patterns of reactivity, we identified 180 bands in sera from gonorrhoea patients. Using a bio-informatics approach we refined the list to 18 bands of interest and identified 13 novel proteins associated with an increase in immuno-reactivity with gonococci.

In summary, we have identified a unique set of gonococcal proteins that have not yet been investigated as potential vaccine antigens and that are the focus of current studies to develop a gonococcal vaccine.

## P198

# Use of short and long read sequencing to investigate genetic relatedness during an outbreak of *E. coli* O157:H7

David Greig[1,2,3], Timothy Dallman[1,2,3], Saheer Gharbia[1], Claire Jenkins[1,2]
[1]National Infection Service, Public Health England, London, United Kingdom. [2]NIHR Health Protection Research Unit in Gastrointestinal Infections, Liverpool, United Kingdom. [3]Division of Infection and Immunity, The Roslin Institute and Royal School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom

## Abstract

We investigated an outbreak of nine cases of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 linked to participation in a mud-based obstacle race. Three additional isolates from cases who could not be linked to the race fell within the same 5 single nucleotide polymorphism (SNP) cluster as the outbreak cases. We used a combination of short-read Illumina and long read Oxford Nanopore Technology (ONT) sequencing data to quantify genetic relatedness of all 12 isolates and to look for micro-evolutionary events in the core and accessory genomes.

Analysis of both the short-read and long-read data placed 10 of the 12 sequences on respective identical nodes on the phylogeny. The systemic errors in ONT base-calling and ambiguous mapping of Illumina reads resulted in variations in the genetic distance when comparing one technology to the other. The variant calling procedure highlighted the importance of masking homologous sequences in the reference genome regardless of which sequencing technology was used.

The genomic content and syntenty of the outbreak isolates was analysed using the Nanopore sequencing data, and three recombination events were identified. These included two prophage-mediated large (1.455Mbp and 0.45Mbp) chromosomal inversions in two samples that were ancestral to the other ten and a 17.8kbp deletion in a single prophage in one isolate.

The ability to characterise the accessory genome in this way is the first step to understanding the significance of these micro-evolutionary events and their impact on the evolutionary history, virulence, and potentially the likely source and transmission of this zoonotic, foodborne pathogen.

**P199**

# AMOEBA & BIOFILMS IN UK CHLORINATED DRINKING WATER DISTRIBUTIONS SYSTEMS: IMPACT ON WATER SAFETY

Gonzalo Del Olmo[1], Geoffrey J. Puzon[2], Carolina Calero Preciado[1], Isabel Douterelo[1]
[1]The University of Sheffield, Sheffield, United Kingdom. [2]CSIRO Land and Water, Floreat, Australia

## Abstract

Amoeba-related diseases have been related with the presence of certain amoebas in domestic water, including drinking water. Biofilms in drinking water distribution systems are able to support amoeba growth by providing a food source and protecting them against disinfectants. Additionally, amoeba growth can be favoured by warm temperatures and climate change appears to contribute to its geographic spread.

The presence of amoeba and its association with potential pathogenic bacteria was studied in a real-scale chlorinated DWDS. The test facility comprised three independent pipe loops fed with water from the local water supply and for this study a varied flow hydraulic profile was applied based on daily patterns observed in real UK distribution networks. The daily regime was repeated for a biofilm growth phase of 30 days. Amoeba viability was tested by a culture-based method, non-nutrient agar (NNA)-E. coli plates, and then confirm by qPCR using specific primers to detect species of amoeba including Naegleria and Acanthamoeba. Amplicon sequencing of the 16s rRNA gene was used to characterise the biofilm and planktonic bacterial communities.

Several amoeba species belonging to the genera Acanthamoeba, Vermamoeba and Naegleria were identified in 30-day old biofilm samples. While the bacterial communities in biofilms were dominated by Variovorax, Pseudomonas and Aquabacterium. This study yielded new insights on the dynamics of amoeba and bacterial communities in DWDS. However, more research is required to accurately establish the impact of these inter-kingdom associations on human health.

## P200

# Polysaccharides of Staphylococcus aureus and Pseudomonas aeruginosa biofilms elicit divergent immune responses via C-type lectin receptors.

Kelly Lee[1], Sonali Singh[1], Yasir Almuhanna[1], Iona Willingham[1,2], Darryl Jackson[1], Miguel Camara[1,2], Luisa Martinez-Pomares[1,2]
[1]University of Nottingham, Nottingham, United Kingdom. [2]National Biofilm Innovation Centre, Nottingham, United Kingdom

## Abstract

ESKAPE organisms (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *enterobacteriaceae*) are important hospital-acquired, opportunistic pathogens that are recognised for extensive antibiotic resistance, lack of effective vaccines and high patient morbidity and mortality. Infection by one or more of these species is observed in patients with underlying conditions, with combinations of *S. aureus* and *P. aeruginosa* characteristic of both the cystic fibrosis lung and severe chronic wounds, and are underpinned by biofilm formation. The biofilm matrix contains, among others, polysaccharides that provide integrity to the 3D structure and may also interact with extracellular DNA and proteins alongside human immune cells via their C-type lectin receptors. *S. aureus* and *P. aeruginosa* biofilms harbour divergent carbohydrate compositions, we therefore hypothesised that the different biofilms will elicit varied immune activities. Here, *S. aureus* biofilm carbohydrate was purified and analysed before performing binding assays using various recombinant CLRs and exposed to primary human immune cells. Also, development of a 3D collagen model has allowed for experiments that are more congruous with chronic wound environments. Previous assays have shown that several lectins bind both *P. aeruginosa* biofilms and purified carbohydrate, and that human dendritic cells interact and change behaviour during incubation. When using *S. aureus*, binding activity and immune response display some differences. Overall, our data has highlighted a divergent immune response to SH1000 and PAO1 biofilms and biofilm products. Ultimately, this work will advance our understanding of biofilm pathogenesis, providing clues towards the development of novel clinical interventions for these infections.

## P201

# A Legionella´s outbreak in a COVID-19 pandemic setting

Catarina Oliveira, Carla Leite, Helena Rodrigues, Fernando Fonseca
Centro Hospitalar Póvoa de Varzim/Vila do Conde, Póvoa de Varzim, Portugal

## Abstract

In this report, the author intends to reinforce the need to test for another potencial causes of pneumonia which present with similar symptoms of SARS-CoV-2 infection and can also be responsible for outbreaks and sometimes fatal, particularly Legionnaires' disease.

*Legionella* bacteria are aerobic, gram-negative, ubiquitous freshwater and soil inhabitants. Pneumonia is the most commonly described manifestation of *Legionella* infection (Legionnaires' disease), acquired through inhalation of aerosolized water droplets containing the bacteria.

*Legionella pneumophila* is the most consistently reported species, being an important cause of severe community-acquired pneumonia that often requires hospitalization and is fatal in approximately 10% of cases. Most cases present sporadically, however, outbreaks can occur when there are appropriate conditions for bacteria to grow and spread, like stagnating water in piping systems in large facilities. Furthermore, the pathogenesis of *Legionella* pneumonia is complex and it is clinically and radiographically similar to other forms of pneumonia.

In the context of pandemic COVID-19, the use of *Legionella* urinary antigen testing continues to be crucial and strongly recommended. Thus, in the outbreak faced in the author's hospital, this screening method proved to be effective and all cases of *Legionella* pneumonia were promptly recognized and notified, allowing public health measures to be taken to prevent the development of other potencial clusters.

## P202

# How to sequence 10,000 bacterial genomes and retain your sanity: an accessible, efficient and global approach

Blanca Perez-Sepulveda[1], Darren Heavens[2], Caisey Pulford[1], Alexander Predeus[1], Ross Low[2], Hermione Webster[1], Gregory Dykes[1], Christian Schudoma[2], Will Rowe[1,3], James Lipscombe[2], Chris Watkins[2], Benjamin Kumwenda[4], Neil Shearer[2], Karl Costigan[1], Kate Baker[1], Nicholas Feasey[5,6], Jay Hinton[1], Neil Hall[2,7], The 10KSG Consortium[8]
[1]University of Liverpool, Liverpool, United Kingdom. [2]Earlham Institute, Norwich, United Kingdom. [3]University of Birmingham, Birmingham, United Kingdom. [4]University of Malawi, Blantyre, Malawi. [5]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [6]Malawi-Liverpool-Wellcome Programme, Blantyre, Malawi. [7]University of East Anglia, Norwich, United Kingdom. [8]Various, Various, United Kingdom

## Abstract

Non-typhoidal *Salmonella* (NTS) are typically associated with enterocolitis and linked to the industrialisation of food production. In recent years, NTS has been associated with invasive disease (iNTS disease) causing an estimated 77,000 deaths each year worldwide; 80% of mortality occurs in sub-Saharan Africa. New clades of *S*. Typhimurium and *S*. Enteritidis have been identified, which are characterised by genomic degradation, altered prophage repertoires and novel multidrug resistant plasmids. To understand how these clades are contributing to the burden and severity of iNTS disease, it is crucial to expand genome-based surveillance to cover more countries, and incorporate historical isolates to generate an evolutionary timeline of the development of iNTS.

We developed and validated a robust and inexpensive method for large-scale collection and sequencing of bacterial genomes. The "10,000 *Salmonella* genomes" project established a worldwide research collaboration to generate information relevant to the epidemiology, drug resistance and virulence factors of Salmonellae using a whole-genome sequencing approach. By streamlining collection of isolates and developing an efficient logistics pipeline, we gathered 10,419 clinical and environmental isolates from collections in low and middle-income countries within six months. Genome sequences are now available for isolates from 51 countries/territories dating from 1949 to 2017, with ~80 % representing African and Latin-American datasets. Our method can be applied to other large sample collections that require maximisation of resources within a limited timeframe. Detailed genome analyses are in progress and it is hoped that the resulting data will contribute to public health control strategies in low and middle-income countries.

## P203

# High-resolution genomic epidemiology of carbapenem-resistant *Acinetobacter baumannii* in an ICU setting

Emma Doughty[1], Haiyang Liu[2], Robert Moran[1], Xiaoting Hua[2], Willem van Schaik[1], Alan McNally[1], Yunsong Yu[2]
[1]University of Birmingham, Birmingham, United Kingdom. [2]Zhejiang University, Hangzhou, China

## Abstract

**Background-** *Acinetobacter baumannii* is a WHO priority pathogen owing to the extent of its antibiotic resistance and threat as a nosocomial infection. Our understanding of specific routes of transmission outside of confined outbreak situations is limited.

**Methods-** We conducted a prospective observational study of carbapenem-resistant *Acinetobacter baumannii* (CRAB) in an intensive care unit (ICU) in Hangzhou, China. Over a 3-month period, we collected 5085 samples from the ICU environment, screening of patients and staff, and clinically-necessary specimens. CRAB were isolated, whole-genome sequenced and analysed using comparative genomics and statistical analysis of sample metadata.

**Results-** CRAB were found in 551 samples: 432/3981 (10.9%) from the environment, 100/976 (10.2%) from screened patients, 0/109 (0%) from staff and 19 from clinical specimens. Multi-locus sequence typing with the Pasteur scheme classified 547/551 (99.3%) CRAB isolates as ST2 or a single nucleotide variant of it, ST187, which likely evolved in the ICU. Population genomic analyses revealed a maximum of 120 core-genome SNPs but a diverse accessory genome amongst these 547 isolates. Spatial and temporal strain co-localisation facilitated horizontal gene transfer events that contributed to this diversity. Isolates could be grouped into strains with different evolutionary histories and epidemiological patterns within the ICU.

**Conclusion-** Multiple CRAB strains were present during this 3-month study, each showing a sporadic, epidemic or possibly endemic epidemiological pattern. Following multiple introductions to the ICU, CRAB strains were transmitted between patients and contaminated bed unit environments across the ward. Analyses revealed transmission patterns to target with appropriate infection prevention and control interventions.

## P204

# Exploring the Cryptic Epidemiological History of Legionnaires' Disease in Scotland Using Whole Genome Sequencing

Jamie Gorzynski[1], Bryan Wee[2], Andrew Smith[3], Ross Cameron[4], Joana Alves[1], Diane Lindsay[3], Ross Fitzgerald[1]
[1]Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom. [2]Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, United Kingdom. [3]Scottish Microbiology Reference Laboratory (SMiRL), Glasgow, United Kingdom. [4]Health Protection Scotland, Glasgow, United Kingdom

## Abstract

*Legionella pneumophila* is the main cause of Legionnaires' disease and a global public health threat. Whole Genome Sequencing (WGS) can help trace environmental origins of *L. pneumophila* infections, providing critical information to guide appropriate interventions.

We investigated the epidemiological history of Legionnaires' disease in Scotland by sequencing all clinical *L. pneumophila* isolates obtained between 1984-2020, and comparing to a sequence dataset of 3211 local environmental and globally representative isolates. To explore epidemiology and establish sources of infection, we employed phylogenetic analysis of core genome multi-locus sequence typing (cgMLST) clusters to measure their diversity and evolutionary relatedness in context with epidemiological metadata.

CgMLST clustering indicated that the majority of infections in Scotland had involved the same endemic clones with a wide temporal and geographical distribution. Among these, phylogenetic analysis revealed nested clades of hospital isolates with evidence of long-term persistence (up to 18 years) from which most nosocomial infections had emerged. Similarly, around 25% of community-associated infections had involved a widely distributed endemic variant (ST37) clone, consistent with enhanced potential to cause disease. Finally, our analysis indicated numerous clusters linked by national or international travel to distinct European regions, revealing cryptic routes of travel-associated *L. pneumophila* infection.

Taken together, our analysis reveals the existence of previously unrecognised endemic clones of *L. pneumophila* that have existed for many years in hospital, community, and travel-associated environments. We propose WGS-based surveillance as a key public health tool for real-time identification of endemic clones with potential for human pathogenicity.

## P205

# Role of Scottish wild deer as a potential source of human pathogenic STEC.

Mairi C. Mitchell[1], Carol Currie[1], Lesley J. Allison[2], Anne Holmes[2], David L. Gally[3], Nadejda Lupolova[3], Margo E. Chase-Topping[4], Sophie Foley[5], Tom N. McNeilly[1]
[1]Moredun Research Institute, Edinburgh, United Kingdom. [2]Scottish E. coli O157/STEC Reference Laboratory, Department of Laboratory Medicine, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom. [3]The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom. [4]Centre for Immunity, Infection and Immunity, University of Edinburgh, Edinburgh, United Kingdom. [5]School of Applied Sciences, Edinburgh Napier University, Edinburgh, United Kingdom

## Abstract

Shiga-toxin producing *E.coli* (STEC) are food-borne pathogens, characterised by production of phage-encoded Shiga toxins (Stx). Cattle, sheep and deer are known to carry STEC, however the role of wild deer in Scotland as a potential source of human STEC infection is unclear. The aim of this study was to investigate the prevalence of STEC O157 in wild deer in Scotland and pathogenic potential of non-O157 STEC deer strains. Faecal samples were collected from 1087 individual deer carcasses between July 2017 and June 2018 and assessed for presence of STEC O157 and *stx* genes by immuno-magnetic separation and PCR, respectively. Sampling was designed to be representative of the annual cull of wild deer entering the food chain, samples were collected from red, roe, sika and fallow species from all regions of Scotland. STEC O157 was present in 3/1087 samples, with overall prevalence of 0.34% (95% CI = 0.02–6.30); 69.5% of samples contained *stx* genes. Of these *stx* gene positive samples 12% were subtype *stx2a*. Modelling of risk factors found *stx2a* presence associated with roe deer, presence of sheep and environmental factors. Whole genome sequence (WGS) data of O157 STEC strains confirmed the presence of *stx2a* and *eae* genes which are associated with severe human disease. Analysis of WGS data from 72 non-O157 STEC strains showed similarity to human clinical strains however, none of the strains were positive for the highly pathogenic *stx2a*:*eae* gene combination. These results indicate that wild Scottish deer are a potential source of human pathogenic STEC strains.

## P206

# Genomic epidemiology of the first London outbreak of antimicrobial resistant sexually transmitted shigellosis

Lewis Mason[1], Timothy Dallman[2], Claire Jenkins[2], Kate Baker[1]
[1]University of Liverpool, Liverpool, United Kingdom. [2]Public Health England, London, United Kingdom

## Abstract

Shigellosis is an intestinal infection caused by Shigella bacteria. Shigella cause an estimated ~200,000 global deaths annually. Antimicrobial resistant (AMR) shigellosis is a significant cause of morbidity in high-income nations, with both multidrug resistant (MDR) and extensively drug resistant (XDR) cases being increasingly reported in Australia, England, and the USA. Sexually transmissible shigellosis was first described in San Francisco, 1974, but it would be a further 30 years before its first description in England. In 2004, London experienced an outbreak of *Shigella sonnei* (*S. sonnei*) mediated sexually transmitted shigellosis, associated with men-who-have-sex-with-men (MSM). Since then, sexually transmissible shigellosis has become endemic in England, with a greater than two-fold increase in Shigella diagnoses within sexual health services from 2015 to 2019. Through genomic exploration of samples from the original 2004 outbreak (provided by Public Health England (PHE)), we identified that the 2004 London outbreak isolates clustered within the base of genotype 3.1, lineage III, a lineage which has since gone on to dominate the global epidemiology of *S. sonnei*. The isolates displayed early evidence of varying degrees of antimicrobial resistance to several drug classes: macrolides, tetracyclines, beta-lactams and sulphonamides. Reconstructing the chronological process of how shigellosis has arrived at its current position in AMR and transmissibility is critical. Further investigation is underway to link this outbreak with MSM-associated shigellosis outbreaks occurring in the early 2000s in other countries to establish whether this lineage globally disseminated; determine the timeframe for global connectivity of shigellosis; and examine the outbreak isolates for virulence determinants.

## P207

# Plasmid cost alleviation by hitchhiking on niche-adaptation

Julia Kloos[1], João A. Gama[1], Joachim Hegstad[1,2], Ørjan Samuelsen[1,3], Pål J. Johnsen[1]
[1]Department of Pharmacy, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway. [2]Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway. [3]Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway

## Abstract

Plasmid maintenance is puzzling because newly acquired plasmids often decrease host fitness. However, host-plasmid association can improve due to compensatory events in either the plasmid or the chromosome. Reshaping the host transcription/translation machinery, through mutations in global regulators, is a common pathway to alleviate plasmid-mediated burdens.

We focused on the amelioration of two unrelated antibiotic-resistance plasmids in a naïve clinical *Escherichia coli* isolate after co-evolution in non-selective media.

Unlike previous works, we did not observe compensatory mutations specific to plasmid-carrying populations. Instead, we observed parallel evolution between plasmid-free and plasmid-carrying populations. Mutations occurred in two groups of genes: *crp*, *cpdA* and *cyaA*, or *arcA/B* – all involved in global regulatory pathways associated with media adaptation. Evolved clones carrying a single SNP in each group of genes were shown to significantly reduce plasmid burden. Thus, deleterious mutations in such genes lead to pleiotropic effects simultaneously allowing niche adaptation and plasmid cost amelioration.

We conclude that niche-adapted plasmid-free bacteria comprise a source of pre-adapted hosts for incoming plasmids.

## P208

# Identification and characterisation of a Novel SXT/R391 ICE mobile genetic element isolated from an Irish wastewater environment.

Michael P Ryan[1,2], Shannon Slattery[1], J Tony Pembroke[1,3]
[1]Department of Chemical Sciences, School of Natural Sciences, University of Limerick, Limerick, Ireland. [2]Applied Science Department, Limerick Institute of Technology, Limerick, Ireland. [3]Bernal Institute, University of Limerick,, Limerick, Ireland

## Abstract

Human and animal pathogenic bacteria are constantly being released into the environment through human activity. Many of these organisms can harbour genes such as virulence genes, antibiotic resistance and heavy metal resistance genes that are inserted into plasmids, transposons and Integrating Conjugating elements (ICEs), leading to potential spread. Such spread can be detected among water and soil communities and in particular in wastewater treatment plants. This makes wastewater and treatment plants a potential reservoir for mobile genetic elements including SXT/R391 ICEs, commonly detected amongst enterobacterial genera. Many plasmid and ICE genomes have been detected serendipitously from clinical sources but few have been identified without selection. Here we examined a domestic wastewater treatment plant to identify, isolate and characterize SXT/R391 ICE's without selection. Standard microbial replica plating in conjunction with ICE specific (conserved integrase gene) PCR techniques were employed to identify an SXT/R391 ICE MGE using a range of enterobactial selective media. A Novel SXT/R391 ICE MGE was identified from a wastewater *Proteus mirabilis* strain. Whole genome sequencing using Ilumina sequencing technology revealed a novel 81 kb element which on annotation contained 75 open reading frames. The "hotspot regions", which contain adaptive genes, encoded a novel bacteriophage defence mechanisms but lacked other selectable determinants. With the continuous arms race between bacteria and phage, bacteria have developed novel resistance mechanism systems that protect the bacteria from phage. Such systems may be key adaptive mechanisms harboured by ICEs particularly in wastewater systems which will contain large phage populations.

## P209

# Oxford Nanopore sequencing of animal group B *Streptococcus* reveals the existence of plasmids shared with human-associated streptococci

Chiara Crestani[1], Taya Forde[1], Mark Holmes[2], Ruth Zadoks[3]
[1]University of Glasgow, Glasgow, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom. [3]University of Sydney, Sydney, Australia

## Abstract

Group B *Streptococcus* (GBS) is a multi-host pathogen with a broad range of hosts and niches. Plasmids are not considered major drivers of GBS evolution, as they are rarely reported in human GBS isolates, with no reports to date from animal GBS. We applied long-read sequencing to a diverse collection of bovine GBS isolates in order to resolve their complete genomes and to identify circular mobile elements.

DNA was extracted from 20 GBS isolates with GenElute Bacterial Genomic Kit. Libraries were prepared with Rapid Barcoding Kit for Oxford Nanopore MinION sequencing, and with Nextera XT Sample Kit and MiSeq Reagent Kit V2 for Illumina. Hybrid assemblies were obtained with Unicycler v0.4.8, resulting in closed circularised genomes.

Three circularised plasmids were identified. Two (pZ2-174 and pZ2-265) were nearly identical to plasmids previously reported in human-associated *Streptococcus dysgalactiae* subsp. *equisimilis* (pW2580) and *Streptococcus pyogenes* (pA996). A high prevalence of pZ2-265 was found using BLASTn among an existing collection of 1,725 GBS assemblies (35% of bovine and 9% of all isolates).

We report the first plasmids detected in bovine GBS, as well as high similarity to plasmids previously detected in streptococcal species that are found in humans but not in cattle. Our data suggest that horizontal genetic exchange occurs among streptococcal species that co-exist within the same environment (e.g. in the human oropharynx) with subsequent potential for transmission to other host species, using multi-host pathogens as a vehicle.This mechanism would greatly expand the potential gene pool that bacterial species can access.

## P210

# Piperacillin/tazobactam resistance in a clinical isolate of *Escherichia coli* due to IS*26*-mediated amplification of $bla_{TEM-1B}$

Alasdair Hubbard[1], Jenifer Mason[2], Paul Roberts[2], Christopher Parry[3], Caroline Corless[2], John van Aartsen[2], Alex Howard[2], Issra Bulgasim[1], Alice Fraser[1], Emily Adams[1], Adam Roberts[1], Thomas Edwards[1]
[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]Liverpool University Hospital Foundation Trust, Liverpool, United Kingdom. [3]Alder Hey Children's NHS Foundation Trust, Liverpool, United Kingdom

## Abstract

We identified a clinical isolate of *Escherichia coli* displaying an unusual, emerging phenotype; piperacillin/tazobactam (TZP)-resistant, 3rd generation cephalosporin-susceptible. Prior to treatment with TZP, a TZP-susceptible *E. coli* isolate was isolated from the same patient. Hyperproduction of a class A β-lactamase has previously been linked to this phenotype, but the mechanism of hyperproduction in isolates lacking promoter region mutations is not well understood.

Clonality of the two isolates was initially assessed with RFLP, and β-lactamase activity was determined using a nitrocefin assay. Both isolates were sequenced on Illumina and Oxford Nanopore Technology platforms and fitness assessed competitively. A plasmid construct containing the insertion sequence IS*26* was used to capture a translocatable unit (TU) *in vitro*.

The two *E. coli* clinical isolates were confirmed to be clonal, with the TZP-resistant isolate hyperproducing $bla_{TEM-1}$. However, no promoter region mutations were identified in the TZP-resistant isolate. Hybrid assembly revealed that an ~11kb segment of DNA was excised from a IS*26* flanked pseudo-compound transposon in the TZP-resistant isolate, forming a circular TU containing $bla_{TEM-1}$. Multiple re-insertion events of the TU, mediated by IS*26*, led to tandem repeats of the TU within the chromosome, increasing the copy number of $bla_{TEM-1}$. Excision and insertion events were confirmed via capture of the TU. Amplification of the TU in the TZP-resistant isolate incurred no significant change in fitness in different environmental conditions.

This study improves the understanding of the TZP-resistant, 3rd generation cephalosporin-susceptible phenotype in *E. coli* and antimicrobial resistance prediction of this phenotype from genotypic data.

**P211**

# Molecular genetics of the virulence plasmid of pathogenic *Escherichia coli* O104:H4.

Rachel Whelan, Gareth McVicker
Nottingham Trent University, Nottingham, United Kingdom

## Abstract

The exchange of mobile genetic elements has previously resulted in the emergence of atypical strains posing a huge risk to human health. One such example was detected in the 2011 European outbreak of *E. coli* O104:H4 which carried an unusual combination of EAEC- and STEC-associated virulence factors on a plasmid and phage respectively. The *E. coli* O104:H4 virulence plasmid has exhibited unusual stability when subjected to a range of environmental stresses, in contrast to frequent plasmid loss *in vivo.* We have investigated the maintenance systems possibly linked to this unique stability, focusing on the toxin-antitoxin (TA) systems responsible for plasmid maintenance and therefore increased virulence. We have analysed the function of putative TA system genes present on the virulence plasmid by inducing their expression from lab-made vectors in order to further understand the mechanisms involved in plasmid maintenance during infection. The disruption of these systems will then be assessed through the application of various environmental stresses, resulting in plasmid loss and therefore the loss of key virulence factors from the cell. The 2011 outbreak strain displayed heightened pathogenicity and provided unforeseen treatment challenges; our experiments therefore provide a model for understanding MGE carriage to predict and combat future outbreaks of similar hybrid pathovars that have arisen through the exchange of mobile genetic elements.

## P212

# Changes in Bacterial Fitness Following the Acquisition of β-Lactamase Encoding Plasmids

Enas Newire[1,2], Adam P. Roberts[3]
[1]University College London (UCL), London, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

## Abstract

The objective of this study was to evaluate the bacterial fitness associated with the acquisition of conjugative plasmids encoding extended spectrum β-Lactamases (ESBL) in *Enterobacteriaceae*.

Conjugation experiments were carried out on filters, using a sodium azide resistant *Escherichia coli* recipient and two, multi-drug resistant donors (clinical isolates of *Klebsiella pneumoniae* and an *E. coli* isolated from Egyptian hospitals). We analysed transconjugants which had acquired $bla_{CTX-M}$-carrying plasmids for fitness and plasmid stability.

Our results show that when a transconjugant has acquired a single IncL/M or IncN plasmid containing $bla_{CTX-M}$ there was a reduction in host fitness of 20% or 24%, respectively. However, when the acquisition of either IncL/M or IncN plasmids was accompanied by other plasmids from the same donor a fitness benefit of 2% or 11%, respectively was observed compared to the recipient. Stability of the IncL/M or IncN plasmids was also increased when acquisition of multiple plasmids occurred. The sequence of the plasmids revealed that the transferable plasmids contained multiple antibiotic resistance genes.

Our results suggest epistatic interactions between the plasmids and between the plasmids and the host which could explain why *Enterobacteriaceae* acquire multiple resistance genes in a stable manner and why these plasmids are able to persist in bacterial populations in the absence of selective pressure.

## P213

# NanoTraDIS: A nanopore-based method for massively parallel analysis of transposon mutant libraries

Muhammad Yasir[1], Keith Turner[1], Martin Lott[1], Steven Rudder[1], David Baker[1], Sarah Bastkowski[1], Andrew Page[1], Mark Webber[1,2], Ian Charles[1,2]
[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom

## Abstract

Transposon insertion sequencing (TIS) is a powerful tool to identify genotype-phenotype associations. All TIS methods described to date use short nucleotide sequence reads to identify insert positions, however these cannot uniquely determine the locations of transposon insertions within repeating sequences whose repeat units are longer than the reads. To overcome this limitation, we have developed a TIS method that uses nanopore sequencing technology to generate long nucleotide sequence reads. We have called this new approach 'Nanopore for Transposon Directed Insertion-site Sequencing' (NanoTraDIS). We have used NanoTraDIS to analyse a large transposon mutant library of *E. coli* BW25113 and generated sequence reads upto14,000 basepairs. This enabled the unique localisation of transposon insertion sites within long repetitive genetic elements of *E. coli*, such as the transposase genes of insertion sequences and the multicopy ribosomal RNA genes. The Oxford Nanopore sequencing device is cheaper than other high-capacity sequencing machines, small and easily portable. NanoTraDIS is also an efficient means of uniquely identifying transposon insertion sites within long repetitive genetic elements, is more cost effective than previous TIS methods, and can be easily transported to, and used in, laboratories that lack access to expensive DNA sequencing facilities. We demonstrate that NanoTraDIS is reproducible, gives comparable results to short read TIS methods for essential genes, and better resolution for repeated regions of the genomes and is amenable to multiplexing. This method provides a new flexible option for TIS sequencing with advantages in cost and working with complex regions of genomes.

## P214

# A *Campylobacter* integrative conjugative element with a CRISPR-Cas9 system targeting competing plasmids: a history of plasmid warfare?

Arnoud van Vliet[1], Oliver Charity[2], Mark Reuter[2]
[1]University of Surrey, Guildford, United Kingdom. [2]Quadram Institute Bioscience, Norwich, United Kingdom

## Abstract

Microbial genomes are highly adaptable, with mobile genetic elements such as integrative conjugative elements (ICE) mediating the dissemination of new genetic information throughout bacterial populations. This is countered by defence mechanism such as CRISPR-Cas systems, which limit invading mobile elements by targetting specific sequences on these elements. Here we have studied the distribution the pVir, pTet and PCC42 plasmids and a new 70-129 kb ICE (CampyICE1) in the foodborne microbial pathogens *Campylobacter jejuni* and *Campylobacter coli*. CampyICE1 contains a degenerated Type II-C CRISPR system consisting of a sole Cas9 protein, which is distinct from the previously described Cas9 proteins from *C. jejuni* and *C. coli*. CampyICE1 is highly conserved in structure and gene order, containing blocks of genes predicted to be involved in recombination, regulation and conjugation. CampyICE1 was detected in 134/5,829 (2.3%) *C. jejuni* genomes and 92/1,347 (6.8%) *C. coli* genomes. Similar ICE were detected in a number of non-jejuni/coli *Campylobacter* species, which lacked a CRISPR-Cas system. Finally, CampyICE1 contained 3 separate short CRISPR spacer arrays, and a total of 124 unique spacers were identified, of which 67 are predicted to target the *Campylobacter* plasmids pVir, pTet, and pCC42, and 12 predicted to target other *Campylobacter* plasmids (63.7%). The presence of a functional CampyICE1 Cas9 protein and matching anti-plasmid spacers was associated with the absence of these plasmids (186/214 genomes), implicating that the CampyICE1-encoded CRISPR-Cas has contributed the exclusion of competing plasmids. Hence the CampyICE1 CRISPR-Cas system may be a part of ongoing plasmid warfare in *Campylobacter* spp.

## P215

# Live or let lyse: Investigation of the arbitrium system in *Bacillus subtilis* phage SPBeta

Aisling Brady[1,2], Nuria Quiles-Puchalt[2], José R Penadés[2]
[1]Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom.
[2]MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, United Kingdom

## Abstract

Temperate bacteriophages can undergo either a lytic or lysogenic lifecycle within their bacterial host cell. Some *Bacillus* phages use a peptide-based communication system, arbitrium, to coordinate lysis-lysogeny decisions as a means of controlling bacterial host populations. In this system the phage produces AimP, a hexapeptide, during the lytic cycle. Once inside the bacterial host cell, AimP reduces expression of the negative regulator of lysogeny, AimX, by binding to the transcription factor AimR, promoting lysogeny. The system has been identified in a wide variety of *Bacillus* phages and conjugative elements however the specific mechanism of action has only been characterised in phage phi3T after infection of the phage.

Here, we study another *Bacillus* temperate phage, SPBeta, that carries a similar arbitrium system to phi3T. However, there are some noteworthy differences. SPBeta encodes a different signalling peptide to phi3T, highlighting the specificity of the system. Additionally, there is no clear *aimX* region. Instead, the phage encodes three sRNAs suggesting a more complex regulation mechanism. To assess the relevance of these differences, and the role of arbitrium in SPBeta biology, we have investigated the two main phage life cycles: infection (lytic) and induction (lysogenic). We show that the presence of AimP leads to a significant reduction in titer following induction of SPBeta prophage. Additionally, deletion of *aimR* results in a similar titer reduction while deletion of *aimP* promotes lysis following induction. Taken together, our results reveal a novel role for the arbitrium system in prophage induction.

## P217

# Mobile genetic elements in an intensive care unit's *Escherichia coli* population

Robert Moran[1], Liu Baomo[2], Emma Doughty[1], Zhuo Chao[2], Alan McNally[1], Willem van Schaik[1]
[1]University of Birmingham, Birmingham, United Kingdom. [2]Guangzhou Medical University, Guangzhou, China

## Abstract

Plasmids and translocatable genetic elements drive the dissemination of antibiotic resistance genes (ARGs). As many studies focus on outbreaks or on specific ARGs and bacterial clones, the diversity and dynamics of ARG-associated elements are not well understood.

Over three months, 118 extended-spectrum beta-lactamase-producing *E. coli* were isolated from patient oral and rectal swabs, staff rectal swabs and from the ward environment in an intensive care unit in Guangzhou, China. The collection was Illumina-sequenced and 19 isolates were Nanopore-sequenced. Over 80 complete plasmid sequences were typed, annotated and used in comparative analyses.

The diverse *E. coli* population contained representatives of 48 sequence types (STs) that carried determinants for CTX-M, CMY, LAP, NDM, OXA and TEM-type beta-lactamases. Most isolates also carried ARGs conferring resistance to other antibiotic classes. ARGs were associated with insertion sequences, transposons or integrons inserted in chromosomal positions, in phage or in plasmids. ARG-associated elements were found in multiple contexts, demonstrating their capacity for dissemination amongst vehicles for horizontal transfer. Isolates carried 2-12 plasmids each with 0-3 per isolate containing ARGs. ARG-containing plasmids ranged from 6-180 kbp and were of various replicon types.

Several replicons were present in multiple STs. In most cases these plasmids shared backbone sequences but clearly diverged from ancestral lineages by acquiring different combinations of translocatable elements. Many plasmid lineages found here are also associated with clinical, agricultural or environmental isolates from various countries. This underlines the multi-faceted nature of ARG dissemination through a complex, heterogenous network of translocatable elements and plasmids circulating globally.

## P218

# Analysis of the target-site preference of the antibiotic resistance encoding conjugative transposon Tn5397

Sarah Alharbi[1,2], Adam Roberts[3], Peter Mullany[2]
[1]Liverpool School of Tropical Medicine (LSTM), Liverpool, UAE. [2]University College London (UCL), London, United Kingdom. [3]Liverpool School of Tropical Medicine (LSTM), Liverpool, United Kingdom

## Abstract

Conjugative transposons (CTns) are discrete DNA segments that can transfer between bacterial cells and often carry antibiotic resistance genes (ARGs). The multi-drug resistant *Clostridioides difficile* strain 630 harbours the tetracycline resistance encoding CTn Tn*5397,* which displays considerable target-site specificity. An important structural feature of the Tn*5397* target-site is the presence of imperfect inverted repeats flanking a central GA dinucleotide (crossover site). The large serine recombinase, TndX catalyze the transposition of Tn5397.

This study aims to investigate the molecular mechanism of Tn*5397* target site selectivity.

Ten mutant target sites, each consisting of 50 bps, were assembled in a single continuous DNA fragment. The mutations were generated at symmetrical positions around the central GA dinucleotide. *In vivo* transposition assays (consisting of a mini-Tn*5397* element and the *tndX* gene, encoding the recombinase, plus the target sites on different replicons) were established in *Escherichia coli*. The insertion of mini-Tn*5397* into the target sites was detected by PCR across the left and the right mini-transposon::target site junctions.

Results confirm successful transposition of mini-Tn*5397* into the wild type target-site and show that the target can tolerate double mutations close to the central crossover site. Also, we identified four positions within the target site that are critical for insertion, where mutations in these positions prevent the insertion of mini-Tn*5397*.

These findings advance our knowledge of Tn*5397* target-site selection and show critical conserved nucleotides within the regions flanking the cross-over site. This work opens up the possibility of altering target-site specificity and using Tn*5397* as a controllable insertional mutagen.

## P219

# Plasmids facilitate pathogenicity, not cooperation, in bacteria

Anna Dewar[1], Joshua Thomas[1], Thomas Scott[1], Geoff Wild[2], Ashleigh Griffin[1], Stuart West[1], Melanie Ghoul[1]
[1]University of Oxford, Oxford, United Kingdom. [2]University of Western Ontario, London, Ontario, Canada

## Abstract

Horizontal gene transfer via plasmids could favour cooperation in bacteria, because transfer of a cooperative gene turns non-cooperative cheats into cooperators. This hypothesis has received support from both theoretical and genomic analyses. In contrast, with a comparative analysis across 51 diverse species, we found that genes for extracellular proteins, which are likely to act as cooperative 'public goods', were not more likely to be carried on either: (i) plasmids compared to chromosomes; or (ii) plasmids that transfer at higher rates. Our results were supported by theoretical modelling which showed that while horizontal gene transfer can help cooperative genes initially invade a population, it does not favour the longer-term maintenance of cooperation. Instead we found that genes for extracellular proteins were more likely to be on plasmids when they coded for pathogenic virulence traits, in pathogenic bacteria with a broad host-range. Taken together, these results support an alternate hypothesis, that plasmid gene location confers benefits other than horizontal gene transfer.

## P220

# Regulatory cascade in SaPI activation

Andreas Haag[1], Magdalena Podkowik[1,2,3], Rodrigo Ibarra-Chávez[1], Francisca Gallego del Sol[4], Geeta Ram[2], John Chen[5], Alberto Marina[4], Richard Novick[2], José Penadés[6,7,8]
[1]Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom. [2]Departments of Microbiology and Medicine, New York University School of Medicine, New York, USA. [3]Department of Food Hygiene and Consumer Health Protection, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland. [4]Instituto de Biomedicina de Valencia (IBV-CSIC) and CIBER de Enfermedades Raras (CIBERER), Valencia, Spain. [5]Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore. [6]University of Glasgow, Glasgow, United Kingdom. [7]Departamento de Ciencias Biomédicas, Universidad CEU Cardenal Herrera, Moncada, Spain. [8]MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, United Kingdom

## Abstract

Staphylococcal pathogenicity islands (SaPIs) are mobile genetic elements encoding superantigens and other toxins and are induced for excision, replication, packaging and intercell transfer by phage-encoded anti-repressors that counter the SaPI master repressor. Though SaPI induction has heretofore been assumed to be the exclusive province of helper phages, we report here the remarkable discovery that one of the SaPIs, SaPI3, can instead be induced only by a second, co-resident SaPI, which must first be induced by a phage. This induction cascade thus represents intricate regulatory triad; SaPI3, the beneficiary of this intracellular largess, is the prototype of a hitherto uniquely immobile SaPI lineage. We report that members of this lineage are controlled by a novel regulatory module and are induced by a highly conserved but previously uncharacterised SaPI protein. SaPI3 and its cousins are thus SaPI satellites, just as most other SaPIs are phage satellites.

## P222

# Should I stay or should I go? Moderation of prophage induction by the Arbitrium system

John Bruce, Edze Westra
University of Exeter, Exeter, United Kingdom

### Abstract

Upon infection of a bacterial host, temperate phage must decide between replication and lysing the host (horizontal transmission), or lysogeny and dormancy as a prophage (vertical transmission). If hosts are plentiful, lysis allows rapid replication through infection of multitudes of sensitive hosts; if hosts are limited or unavailable, lysogeny allows much slower replication as a prophage and the opportunity to lyse the host in future. Uncertain host availability should favour temperate phage that can track changes in host availability and adjust their transmission decisions accordingly. SPbeta phage use the Arbitrium system to respond plastically to changes in host availability. These phage produce signalling molecules during infection which accumulate and cause phage to switch from lysis to lysogeny as available hosts are diminished. However, upon lysogeny, temperate phage have another decision to make: when to revert to horizontal transmission and lyse the host. Here, we show that SPbeta phage also use the Arbitrium system to make informed decisions over prophage induction. We demonstrate that signal molecules produced during infection are durable, and even low signal concentrations act to maintain the prophage state through silencing of phage lysis machinery. Avoiding induction when signal is present, indicative of recent infections and limited host availability, may allow SPbeta phage to continue transmitting vertically as a prophage until opportunities for horizontal transmission arise.

## P223

# Human Cytomegalovirus infection among treatment-naive HIV-1 infected patients in Ethiopia

Mulugeta Kiros[1], Alene Geteneh[2], Henok Andualem[1], Derbie Alemu[3], Abebech Tesfaye[4], Dessalegne , Abeje Tefera[4], Adane Mihret[4], Dawit Hailu Alemayehu[5], Andargachew Mulu[4]
[1]Debre Tabor University, Debre Tabor, Ethiopia. [2]Woldia University, Woldia, Ethiopia. [3]Arba Minch College of Health Sciences, Arba Minch, Ethiopia. [4]Armauer Hansen Research Institute, Addis Ababa, Ethiopia. [5]Armauer Hansen Research Institute, Addis Ababa, Afghanistan

## Abstract

Subclinical human cytomegalovirus (HCMV) replication is associated with immune dysfunction in immuno-suppressed antiretroviral therapy (ART) naive HIV infected individuals. No data is documented in Ethiopia so far concerning HCMV co-infection among HIV infected individuals. Hence, this study was aimed at generating data regarding the prevalence of active HCMV infection among treatment-naive HIV-infected individuals from Ethiopia. For this purpose, we enrolled 97 treatment-naive HIV infected study subjects in Addis Ababa from June to December 2018. ELISA and conventional PCR were performed consecutively to detect HCMV specific IgM antibody and HCMV DNA respectively. Of the 97 study subjects, 12 (12.4 %) were positive for anti-CMV IgM antibodies but were not confirmed by PCR. With regard to the PCR positivity, 4/97 (4.1 %) samples were positive for HCMV DNA. No statically significant associations were found between the dependent and independent variables. The presence of HCMV DNA in the current study highlights the need for a routine laboratory diagnosis for preventing HCMV disease among HIV-infected individuals early. Besides, the use of anti-CMV therapy for these CMV viremic individuals is also recommended as this can reduce the burden of CMV complications and consecutively prolonging the life of HIV infected individuals.

## P224

# Characterization of a persistent baculovirus infection established in an insect cell line

Raquel Arinto-Garcia[1,2], Carina Bannach[1,2], Daniel Leite[2], Linda King[2], Robert Possee[1]
[1]Oxford Expression Technologies Ltd, Oxford, United Kingdom. [2]Dept. Biological & Medical Sciences, Oxford Brookes University, Oxford, United Kingdom

## Abstract

Insect baculoviruses often cause a lethal infection of their larval host. However, many species harbour persistent, non-lethal infections that are rarely observed *in vitro*. In 2011, a serendipitous event led to the establishment of a persistent infection in *Trichoplusia ni* (Hi5) cells with an Autographa californica multiple nucleopolyhedrovirus (AcMNPV) mutant (AcUW1.*lacZ*), where the very late *p10* gene had been replaced by the bacterial β-galactosidase coding region. From this culture, a clonal cell line (C20) was established that has been in continuous culture to the present day. The C20 cells have remarkable resilience; surviving without passage or addition of fresh medium for over a month. Very low levels of budded virus (BV) are generated, although these can amplify normally when used to inoculate other susceptible cell lines (e.g. Sf9). While late passage virus from C20 cells exhibits several significant genome insertions and deletions, these are not seen in virus isolated from early cultures of persistently-infected cells. This suggests a rapid response of the Hi5 cells to virus infection that results in a state of semi-resistance. Furthermore, C20 cells are largely resistant to superinfection with AcMNPV. Microscopy studies indicated that superinfection may be blocked at the virus adsorption and uptake stages, suggesting changes in the cell cytoskeleton. Whole cell transcriptome analysis of C20 cells showed that all virus genes in the persistent infection were expressed, but without the characteristic early-late-very late phases. Analysis of host cell transcripts provided evidence that some pathways were upregulated in the persistently-infected cells while others were downregulated.

## P225

# Use of Equine Herpesvirus type 1 glycoprotein pseudotyped lentiviral particles for the development of serological tests and assessment of lyophilisation for transport and storage

Cecilia Di Genova[1], Gabrielle Sutton[2,3], Romain Paillot[4], Nigel Temperton[1], Stéphane Pronost[2,3], Simon D Scott[1]
[1]Viral Pseudotype Unit, Medway School of Pharmacy, University of Kent, Chatham Maritime, United Kingdom. [2]LABÉO Frank Duncombe, Saint-Contest, France. [3]NORMANDIE UNIV, UNICAEN, BIOTARGEN, Caen, France. [4]Writtle University College, Chelmsford, United Kingdom

### Abstract

Equine herpesviruses (EHVs) are enveloped DNA viruses predominantly infecting members of the Equidae family. EHVs primarily cause respiratory disease, however EHV-1 can produce cases of a neurological disease, abortion and neonatal death. Thus, these viruses represent a welfare issue for the equine industry and scientific focus for researchers. EHV-1 exhibits a complex array of 12 glycoproteins on its surface envelope, but it is unclear precisely which are important for virus cell entry and the role of each in host immune response. In order to investigate the contribution of these glycoproteins, pseudotype viruses (PVs) could provide a useful study tool. We have successfully generated functional EHV-1 pseudotyped lentiviruses bearing four glycoproteins, gB, gD, gH and gL (sequences derived from an aborted foetus during a large EHV1 outbreak strain in Normandy, France). PVs were employed in a pseudotype virus neutralisation test (PVNT) to measure levels of specific neutralising antibodies serum samples (n=52) taken longitudinally from experimentally infected ponies, compared with uninfected controls.

PVs routinely require -80oC for long term storage and a dry ice cold-chain during transport which can impede dissemination and utilisation in other laboratories. Consequently, we further investigated whether freeze-drying (lyophilisation) of EHV-1 PV could address this issue. PVs were lyophilised and pellets either reconstituted immediately or stored under various temperature conditions, sampling at different timepoints. The recovery and functionality of these lyophilised PVs was compared with standard frozen aliquots in titration and neutralisation tests.

## P226

# HCMV UL148 and UL148D regulate multiple immune pathways by impairing expression of ADAM17

Anzelika Rubina[1], Mihil Patel[1], Katie Nightingale[2], Ceri Fielding[1], Simone Forbes[1], Betty Lau[3], Luis Nobre[2], Kelly Miners[1], David Price[1], Dawn Roberts[1], Virginia-Maria Vlahava[1], Borivoj Vojtesek[4], Andrew Davison[3], Michael Weekes[2], Gavin Wilkinson[1], Richard Stanton[1], Peter Tomasec[1], Eddie Wang[1]
[1]Cardiff University, Cardiff, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom. [3]University of Glasgow, Glasgow, United Kingdom. [4]Masaryk Memorial Cancer Institute, Brno, Czech Republic

## Abstract

Human cytomegalovirus (HCMV) is one of the most widespread, highly successful herpesviruses, establishing a life-long viral infection in humans. HCMV has been described as a paradigm of immune evasion able to manipulate many immune functions in the host. Here, we describe a novel, post-translational mechanism in which HCMV downregulates a disintegrin and metalloproteinase 17 (ADAM17), a 'sheddase' that cleaves and releases over 80 membrane-anchored cytokines, cell adhesion molecules and other receptors. A screen of HCMV deletion mutants identified UL148 and UL148D as the HCMV genes responsible for ADAM17 downregulation, working synergistically to alter ADAM17 levels in infected cells. We demonstrate that UL148/UL148D interfere with ADAM17 maturation, resulting in expression of only the intracellular immature precursor, and absence of mature ADAM17 on the surface of wildtype HCMV-infected cells. The consequences of ADAM17 downregulation by HCMV were analysed using proteomics and validated using biochemical and flow cytometric techniques, revealing impact on multiple cell surface and secreted host proteins. This included stabilisation of surface TNF Receptor 2, as well as Vasorin and Jagged1, which have recognised roles in Treg development. Other known ADAM17 targets were not stabilised, suggesting specific control by HCMV. Vaccinia virus, another paradigm of immune evasion, also impaired surface ADAM17 expression, suggesting that manipulation of ADAM17 may represent a novel immunoregulatory hub targeted by large DNA viruses.

## P227

# 3D co-cultures of epithelial cells with immune cells as a model of HSV and oncolytic HSV infections

Ilaria Epifano[1], Joe Conner[1], Iain A. McNeish[2], Sheila V. Graham[1]
[1]College of Medical Veterinary and Life Sciences, Institute of Infection Immunity and Inflammation, MRC-Centre for Virus Research, University of Glasgow, Glasgow, United Kingdom. [2]Division of Cancer, Department of Surgery and Cancer, Imperial College, London, United Kingdom

## Abstract

Virus infectivity is commonly investigated with in vitro monolayer cell cultures or *in vivo* animal models. Ease of growth and manipulation and low cost characterise standard cell culture. Animal models allow investigation of infectivity in the context of tissue structure and environment but are costly and can be limited by species variations. Neither approach can recapitulate the human context, the tissue microenvironment and human immune components. Three-dimensional organotypic raft tissue models can provide most of the advantages of in vitro and in vivo models.

We successfully established HSV and oncolytic HSV (HSV1716) infection in 3D raft cultures of epithelial non-tumour (HaCaT) and tumour (SiHa, OVCAR3 and TOV21G) cell lines. Our 3D models allowed the evaluation and quantification of virus replication and the recovery of the virus both in culture media and tissues.

We developed a complex 3D co-culture of epithelial cells with human immune cells in order to mimic the tissue microenvironment. This innovation allowed us to study the effect of immune cells in cell killing by HSV1716 in the in vitro tissues. In HSV1716-infected co-culture tissues, immune cells were identified throughout the tissue and some migrated to the areas of infection. The immune activity was identified through increased IL-8 release. Moreover, combining infection with immune cell infiltration increased tumour cell killing in the 3D co-culture model. This new co-culture model could be further developed to identify the role of immune cells in oncolytic viroimmunotherapy and to dissect the involvement of specific single immune cell subpopulations.

## P228

# Novel Insights into the Roles of Bcl-2 Homolog Nr-13 (vNr-13) Encoded by Herpesvirus of Turkeys in the Virus Replication Cycle, Mitochondrial Networks, and Apoptosis Inhibition

Vishwanatha Reddy, Yashar Sadigh, Na Tang, Yongxiu Yao, Venugopal Nair
The Pirbright Institute, Woking, United Kingdom

### Abstract

The Bcl-2 (B cell lymphoma 2)-related protein Nr-13 plays a major role in the regulation of cell death in developing avian B cells. With over 65% sequence similarity to the chicken Nr-13, herpesvirus of turkeys (HVT) vNr-13, encoded by the HVT079 and HVT096 genes, is the first known alphaherpesvirus-encoded Bcl-2 homolog. HVT-infected cells were reported to be relatively more resistant to serum starvation, suggested that vNr-13 could be involved in protecting the cells. Here, we describe CRISPR/Cas9-based editing of exon 1 of the HVT079 and HVT096 genes from the HVT genome to generate the mutant HVT-Δ*vNr-13* to gain insights into its functional roles. Overall, wild-type HVT and HVT-Δ*vNr-13* showed similar growth kinetics; however, at early time points, HVT-Δ*vNr-13* showed 1.3- to 1.7-fold-lower growth of cell-associated virus and 3- to 6.2-fold-lower growth of cell-free virus. In transfected cells, HVT vNr-13 showed a mainly diffuse cytoplasmic distribution with faint nuclear staining. Further, vNr-13 localized to the mitochondria and endoplasmic reticulum (ER) and disrupted mitochondrial network morphology in the transfected cells. In the wild-type HVT-infected cells, *vNr-13* expression appeared to be directly involved in the disruption of the mitochondrial network, as the mitochondrial network morphology was substantially restored in the HVT-Δ*vNr-13*-infected cells. IncuCyte S3 real-time apoptosis monitoring demonstrated that vNr-13 is unequivocally involved in the apoptosis inhibition, and it is associated with an increase of PFU, especially under serum-free conditions in the later stages of the viral replication cycle. Furthermore, HVT blocks apoptosis in infected cells but activates apoptosis in noninfected bystander cells.

## P229

# Characterisation of novel secreted antiviral factors to HCMV

Martin Potts[1,2], Robin Antrobus[2], Micahel Weekes[2], Mark Wills[1]
[1]Department of Medicine, University of Cambridge, Cambridge, United Kingdom. [2]Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom

## Abstract

Human cytomegalovirus (HCMV) infection induces a diverse cellular immune response, including NK cells and polyfunctional $CD4^+$ and $CD8^+$ T-cells. However, the differential contribution of these distinct immune cell populations to viral surveillance and the composition of the secreted factors (secretomes) that they produce remain poorly characterised. Using an *in vitro* viral dissemination assay, we have shown that isolated $CD4^+$ and $CD8^+$ T-cells are capable of controlling HCMV dissemination, recapitulating their important role *in vivo*. Furthermore, the secretomes produced by these cell populations when co-cultured with HCMV-infected fibroblasts are highly antiviral and restrict HCMV dissemination *in vitro*. Initial secretome analysis indicates that the observed antiviral activity is accounted for in part by the action of IFNγ and TNFα but independent of type I and type III IFN activity.

To determine the composition of these antiviral secretomes using an unbiased quantitative approach, we developed a multiplexed proteomic method using a combination of both SILAC and tandem mass tag (TMT) labelling. This has enabled analysis of secretomes produced by immune cells co-cultured with HCMV-infected fibroblasts from different donors in a single experiment and determination of the cellular origin of each secreted protein. Using this method we have generated a detailed characterisation of the secretome produced by whole PBMC in order to model an *in vivo* immune response. Application of this technology has provided a better understanding of the complex immune response raised against HCMV. Expanded profiling of isolated immune cell populations will enable further dissection of secreted antiviral immunity.

## P230

# Separating small extracellular vesicles from baculovirus virions

Lex Van Es[1,2], David Carter[1], Robert Possee[2], Linda King[1]
[1]Oxford Brookes University, Oxford, United Kingdom. [2]Oxford Expression Technologies Ltd, Oxford, United Kingdom

## Abstract

Extracellular vesicles (EVs) are key messengers between cells, transporting proteins and RNA to recipient cells which may alter their actions. Small EVs of cells infected with HIV or herpes simplex virus, among others, were found to illicit pro- or antiviral reactions in non-infected cells. It would be interesting to study if small EVs play a similar role in insect-virus interactions, which can be studied in the baculovirus infection in *Spodoptera frugiperda*. Firstly, to identify the effect of small EVs from infected cells upon naïve cells, it is essential to separate the virions from small EVs. To this end, size exclusion chromatography was combined with a two layer sucrose density gradient ultracentrifugation.

Size exclusion chromatography separated small EVs and virions from proteins and other small molecules. Various sucrose concentrations and ultracentrifugation times were tested. Nanoparticle tracking analysis showed two particle peaks, located at the interphase between the two sucrose gradients and at the top of the gradient. Plaque assay and qPCR analyses showed the vast majority of virions were concentrated at the interphase. Western blot for a major viral glycoprotein showed it was abundant at both particle peaks. TEM analysis will be used to confirm the presence of small EVs, expected to be concentrated at the top of the gradient. Together these results indicate these methods are able to isolate small EVs from the vast majority of the baculovirus virions. Provided the size and density are not too close, these methods may be optimised to separate other virions from small EVs.

## P231

# DNA-PK senses DNA virus infection in human cells

Dayana Hristova, Marisa Oliveira, Emma Wagner, Brian Ferguson
University of Cambridge, Cambridge, United Kingdom

### Abstract

The type I interferon (IFN-I) response to virus infection is initiated by the detection of nucleic acids by intracellular pattern recognition receptors (PRRs). The sensing of DNA viruses by PRRs is carried out by a number of DNA-binding PRRs that signal via the adaptor protein stimulator of interferon genes (STING) to drive IFN-I transcription. We previously described the role of DNA-dependent protein kinase (DNA-PK) as a viral DNA sensor in murine fibroblasts. In this study we show that DNA-PK is essential for the host response to DNA and DNA viruses in human fibroblasts. In the absence of the catalytic subunit of the DNA-PK heterotrimer, DNA-PKcs, fibroblasts are deficient in their ability to activate a IFN-I response to DNA. DNA-PKcs is activated rapidly following exogenous DNA transfection or DNA virus infection and is required for signalling via STING and the kinase TBK-1 to the transcription factor interferon regulator factor 3 (IRF-3). Most wild-type DNA viruses combat intracellular DNA PRRs using immunomodulatory proteins encoded in their genomes and are effective at blocking IFN-I responses in infected cells. Here we make use of attenuated vaccina and herpes simplex 1 viruses that are lacking the immunomodulators that target DNA sensing mechanisms. We show that DNA-PKcs can sense these viruses and is required for triggering the IFN-I response in infected cells. These data cement the role of DNA-PK in the sensing of DNA virus infections in human cells.

## P232

# The role of TTC4 and HSP90 in Bovine Alphaherpesvirus Type 1 Replication

Beth Thompson, Inga Dry, Enguang Rong, Colin Sharp, Eleanor Gaunt, Spring Tan, Bob Dalziel
Roslin Institute, Midlothian, United Kingdom

## Abstract

Bovine alphaherpesvirus type 1 (BoHV-1) causes Infectious Bovine Rhinotracheitis (IBR) and genital tract infections, associated with significant production losses in the cattle industry. A genome wide CRISPR Knock Out (GeCKO) screen performed by our group identified the gene encoding tetratricopeptide repeat protein 4 (TTC4) protein as proviral during BoHV-1 replication. The role of TTC4 in viral replication has not previously been investigated; however, it is a co-chaperone to Heat Shock Protein 90 (HSP-90) which plays a key role in the replication of other alphaherpesviruses including HSV-1. In this project we seek to characterise the role of TTC4 and HSP90 during BoHV-1 replication.

TTC4 has been validated as proviral for BoHV-1 using CRISPR knockdown. Late BoHV-1 protein production is completely blocked by treatment of susceptible cells with geldanamycin, a HSP90 inhibitor, indicating that, as expected, both proteins are important for optimal virus replication. Subsequent work will use CRISPR-Cas9 Mediated gene editing in bovine kidney MDBK cells to further assess the effect of TTC4 knockout on BoHV-1 replication. To assess whether the proviral effects of TTC4 and HSP90 rely on interactions between these two proteins, site specific mutagenesis of a TTC4 overexpression construct will be used to abolish the interaction of HSP90 and TTC4.

Control of BoHV-1 relies on good husbandry, vaccination, and treatment of secondary bacterial infections. HSP90 inhibitors are effective broad-spectrum antimicrobials in-vitro, but their low therapeutic index precludes their clinical use. Establishing a role for TTC4 in BoHV-1 replication may identify it as a potential alternative therapeutic target.

## P233

# New role of vaccinia virus protein C4: association with the cytoskeletal protein filamin-b

Iliana Georgana[1], Simon R. Scutts[1,2], Chen Gao[1], Hongwei Ren[1,3], Edward Emmott[1,4], Jinghao Men[1], Keefe Oei[1], Geoffrey L. Smith[1]
[1]Department of Pathology, University of Cambridge, Cambridge, United Kingdom. [2]Walter and Eliza Hall Institute, University of Melbourne, Melbourne, Australia. [3]Imperial College London, London, United Kingdom. [4]University of Liverpool, Liverpool, United Kingdom

## Abstract

Vaccinia virus (VACV) is a large DNA virus that dedicates the vast majority of its proteins in modulating the host's immune response. VACV protein C4 is an immunomodulator, that has been previously found to inhibit the activation of both the NF-κB signalling cascade and the DNA-PK-mediated DNA sensing pathway. Both roles are mediated via its C-terminal region, whereas its N-terminus has not been associated with any function and has a hitherto unknown role. Here we show that C4 N-terminal region contributes to virulence *in vivo*, when expressed alone during infection, and additionally, it interacts with filamin-b (FLNB), a cytoskeletal protein. C4 associates with the C-terminus of FLNB and this interaction has been found to be direct by analytical size-exclusion chromatography. FLNB is a large actin-binding protein, which is implicated in cellular processes by acting as a scaffold for signalling molecules, mainly involved in cell motility and growth. Deletion of FLNB from the cells resulted in bigger plaque size and increased viral yield, indicating that FLNB is a restriction factor for VACV infection. These data demonstrate that VACV protein C4 has a new function, which contributes to virulence and which engages the cytoskeleton. Furthermore, we see here that the cytoskeleton performs further, previously uncharacterised functions during VACV infection.

## P234

# Understanding the viral diversity of Hepatitis B virus in Saudi Arabia using Next Generation Sequencing (NGS)

AHMED ALESSA[1], Arvind Patel[1], Daniel Mair[1], Sreenu Vattipally[1], Ana Da Silva Filipe[1], Tamer Abdelrahman[2]
[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [2]Laboratoire National de Sante, Dudelange,, Luxembourg

## Abstract

**Aims: U**nderstanding viral diversity of HBV in Saudi and development of an in vitro model for antiviral screening. We developed protocols for HBV full genome sequencing using NGS that will help to inform the public health plan for eradication in the kingdom.

**Method:** Plasma samples with known viral load were collected retrospectively from two hospitals in Saudi. DNA samples were extracted using MagNA Pure LC® kit. Sequencing were conducted using the illumina MiSeq® platform. Later, we designed target sequence capture panel from our dataset of metagenomics sequences that already been constructed from the output and we validated a new sequencing strategy using Nimbelgen target enrichment (Roche).

**Results:** 100 patient's sample with chronic hepatitis B infection were enrolled in this study. We sequenced viral DNA using metagenomic approach to design target enrichment protocol. Construction of a phylogenetic tree incorporating our sequences and other published HBV sequences showed that the sequences are derived from the same evolutionary tree. Metagenomic data enabled us to design the target enrichment protocol that allow large-scale sequencing of all samples. Coverage of 100 % was observed for all reads. The results suggested that HBV genotype D is predominant in Saudi.

**Conclusion:** We are covering a region that was not covered by any study . Data showed that NGS is a reliable tool for genotype prediction that makes it a useful tool for clinical evaluation while an alternative method might be needed to confirm other genotypes. Also, we have created HBV Sequence database for identifying resistance-associated variants to inform any future treatment . The database will help national guidelines for eradication of HBV in Saudi.

## P235

# Controlling the lytic switch; can m6A-modified RNA be used as an Anti-Viral Target?

Amy Barker[1], Chris Fullenkamp[2], Konstanin Roder[3], Samuela Pasquali[4], Jay Schneekloth[2], Adrian Whitehouse[1]
[1]University of Leeds, Leeds, United Kingdom. [2]NCI, Frederick, USA. [3]University of Cambridge, Cambridge, United Kingdom. [4]Universite Paris Descartes, Paris, France

## Abstract

KSHV has a biphasic life cycle encompassing a latent state and lytic replication. The KSHV replication and transcription activator viral protein, encoded from open reading frame 50 (ORF50), is the key viral protein which drives the switch between the latent and lytic phases (Guito and Lukac, 2012). We have recently demonstrated that KSHV manipulates the host cell N6-methyl adenosine ($m^6A$) RNA modification pathway to enhance viral gene expression. Specifically, we have shown that the KSHV ORF50 transcript is $m^6A$ methylated, allowing the recruitment of the $m^6A$ reader protein, Staphylococcal nuclease domain-containing protein 1 (SND1), resulting in the stabilisation of the ORF50 transcript and efficient KSHV lytic replication (Baquero-Perez et al. 2019).

Further analysis of the m6A modified site with the ORF50 transcript has identified an RNA stem-loop, termed ORF50-1, which is a $m^6A$-modified 43-mer, essential for SND-1 binding, thought to occur in a secondary structure/ sequence-dependent manner.

Generating *in silico* 3D structures of the ORF50-1 RNA in its native 'N' and m6A-modified 'M' form confirms that the presence of the m6A-modification has repercussions in the lower part of the stem-loop and predicts a different secondary structure (collab. Pasquale and Roeder).

Due to the obvious structural differences anticipated, RNA-binding ligands have been identified using Small Molecule Microarrays (SMMS) (collab. Fullenkamp and Schneekloth). By investigating these ligands in biophysical experiments, we have verified RNA-binding and optimised cell-based assays to assess anti-viral properties. It is hoped these experiments will highlight the importance of A versus $m^6A$ within the lytic phase of KSHV's lifecycle.

## P236

# The 627-domain of PB2 is required for a direct interaction between the influenza A virus polymerase and cellular GTPase Rab11a

Hana Veler[1], Haitian Fan[1], Loïc Carrique[1], Jeremy Keown[1], Jonathan M Grimes[1,2], Ervin Fodor[1]
[1]The University of Oxford, Oxford, United Kingdom. [2]Diamond Light Source Ltd, Didcot, United Kingdom

## Abstract

The influenza A virus (IAV) genome consists of eight viral RNA (vRNA) segments assembled into ribonucleoprotein complexes (vRNPs) containing PA-PB1-PB2 heterotrimeric RNA-dependent RNA polymerase (RdRP) and nucleoprotein. Upon successful replication in the cell nucleus, vRNP complexes are exported from the nucleus and transported across the cytoplasm to be packed into progeny virions. The GTPase Rab11a associated with recycling endosomes is believed to contribute to this process by mediating the cytoplasmic transport of vRNPs. However, how vRNPs interact with Rab11a remains poorly understood.

Recombinant Rab11a and RdRP were expressed in *E. coli* and insect cells, respectively, and purified by affinity chromatography and gel-filtration; their interaction was investigated using pull-down assays. Rab11a was found to interact with RdRP directly, suggesting that RdRP mediates the interaction between vRNPs and Rab11a. To determine which domains of the RdRP are important for its interaction with Rab11a we deleted either the 627-domain alone or together with the PB2 cap-binding and mid-link domains. Both mutants failed to bind to Rab11a, indicating that the 627-domain of PB2 is required for the interaction. We then expressed and purified the PB2 C-terminus (PB2-C) from human and avian IAV and showed that avian PB2-C fails to form a complex with Rab11a, suggesting that the interaction between RdRP and Rab11a might be species-specific.

Our work provides evidence that the IAV RdRP interacts directly with Rab11a and that the 627-domain of the PB2 subunit is involved in the interaction. This finding expands our understanding of the cytoplasmic transport of the IAV vRNPs.

## P237

# Investigating alternative AUG usage in avian influenza A virus segment 2

Elizabeth Billington[1,2], Rute Maria Pinto[3], Sam Lycett[2], Holly Shelton[1], Paul Digard[2]
[1]The Pirbright Institute, Pirbright, United Kingdom. [2]The Roslin Institute, Edinburgh, United Kingdom.
[3]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

## Abstract

Influenza A viruses (IAV) have a segmented, negative sense RNA genome. PB1-F2 is an IAV accessory protein encoded by segment 2, in the +1 reading frame. Avian IAVs predominantly encode full length PB1-F2s, whereas human IAVs often have stop codons resulting in C-terminus truncations or ablation of PB1-F2 expression. One reported function of PB1-F2 is innate immune antagonism, which requires C-terminal motifs. Full length PB1-F2 is translated from AUG 4 of segment two, however there are often one or more in frame downstream AUGs (AUGs 7, 8, or 9).

Bioinformatic analysis of avian IAV segment 2 sequences indicates conservation of open reading frames encoding the PB1-F2 C-terminus, despite some subtypes acquiring N-terminal stop codons that persist through several years. Conservation of C-terminal AUGs 7-9 leads to the hypothesis that these serve as independent initiation codons for the C-terminus. We show C-terminal fragment expression from specific AUG codons in 293T cells using tagged proteins.

We generated segment 2 mutants in an avian H5N1 IAV background, which differed in the presence or absence of the AUG start codons or stop codon positions in PB1-F2. Significant differences in viral polymerase activity, measured using mini-replicon assays, were observed for some stop codons, but none of the AUG mutants. However, mutating AUG9 in the H5N1 background resulted in a significantly faster growth kinetics following low MOI infections. We continue to address whether the C-terminus of PB1-F2 has corresponding functions to the full length PB1-F2 protein and its impact on viral fitness in avian hosts.

## P238

# The WD domain of ATG16L1 is a novel barrier to infection by influenza virus.

Benjamin Bone[1], Matthew Jefferson[1], Alina Rozanova[2], Yohei Yamauchi[2], Tom Wileman[3,1], Penny Powell[1]
[1]University of East Anglia, Norwich, United Kingdom. [2]University of Bristol, Bristol, United Kingdom.
[3]Quadram Institute, Norwich, United Kingdom

## Abstract

**Background:** ATG16L1 is a vital component in LC3 lipidation to endosomes, with its WD domain being necessary for the innate immune responses of LC3 associated phagocytosis/endocytosis (LAP/LANDO) (Fletcher *et al.* 2018). Recently, we have shown that removal of the WD domain increases the severity of influenza A virus (IAV) infection in mice (Wang et al. 2020). ATG16L1 has other non-canonical roles, such as in intracellular cholesterol transport, shuttling it to the plasma membrane (PM) (Tan *et al.* 2018). This study investigates the role of the WD domain in the uptake of IAV into cells *in vitro*.

**Results:** We investigated IAV replication and the antiviral response in mouse embryonic fibroblasts (MEFs) derived from a mouse lacking the WD domain (ΔWD) of ATG16L1. We showed that there was an increased replication of IAV at 24-72 hpi in ΔWD MEFs as well as an increase in antiviral cytokine expression. We investigated whether this was due to increased virus entry into cells. Using assays for cell entry and fusion, we showed that IAV could gain access to the cell faster in ΔWD MEFs. When IAV fusion was forced at the PM of ΔWD MEFs at a low pH we found that IAV entered cells faster, indicating differences in the membrane composition at the PM which may explain the increased uptake of IAV in ΔWD MEFs. We are investigating these differences in PM composition using a variety of techniques. Our results suggest that the WD domain of ATG16L1 is a novel barrier to incoming IAV.

## P239

# Quantification of Maternally-Derived Chicken Astrovirus Antibodies in Eggs

Kerry McIlwaine[1], Victoria Smyth[2], Kenneth Lemon[2], Irene Grant[1]
[1]Queen's University Belfast, Belfast, United Kingdom. [2]Agri-Food and Biosciences Institute, Belfast, United Kingdom

## Abstract

White chicks hatchery disease has emerged within the past decade worldwide resulting in high numbers of embryo deaths and weak, runted broiler chicks with pale plumage, which typically die within 24 hours. Economic losses due to this disease are high costing Canadian producers over US $12,000 per 10,000 hens. The causative agent of this disease was identified as a specific strain of chicken astrovirus (CAstV) belonging to the CAstV Biv subgroup. While the majority of circulating CAstV strains appear to be of low or no pathogenicity, other CAstV strains are associated with a fatal kidney disease in young chickens and a second hatchery disease leading to a high proportion of culled chicks, the strains belonging to subgroups Biii and Bi, respectively. Although CAstVs are typically transmitted horizontally between birds, vertical transmission has been demonstrated in these cases and therefore a breeder vaccine for hens that protects against all B group CAstV infections is highly desirable to protect chicks.

Fifteen in-lay, specific pathogen free hens, aged 32 weeks and tested free of CAstV infections by RT-PCR and ELISA, were vaccinated with two oral doses and one intramuscular dose of live, purified CAstV (Bi strain 11672) prototype vaccine containing 5 x $10^4$ virions/dose. A study was conducted to compare two methods of antibody purification from egg yolk and quantified using the CAstV ELISA. Eggs containing maternally-derived antibodies were collected from the hens and the levels of antibodies present compared with those circulating in the hens.

## P240

# Understanding the Role of Norovirus VP1 in Viral Infectivity and Persistence

Jake Mills, Joseph Snowden, Morgan Herod
University of Leeds, Leeds, United Kingdom

## Abstract

Human noroviruses (HNV) are a prevalent cause of gastroenteritis that contribute to >200,000 deaths each year and cost >£40 billion worldwide per annum. There is currently no approved vaccine or therapy, and a greater understanding of the virus life-cycle could help develop new approaches towards disease control. Although HNV infection is usually self-limiting, persistent infections can establish in immunocompromised people - however the underlying mechanisms are poorly understood. Our studies use the murine norovirus (MNV) model system to investigate fundamental virus biology, and several strains of MNV can also persist in the murine host. The primary receptor for MNV, CD300lf, interacts with a network of amino acids (AAs) on the protruding domain of the virus major capsid protein (VP1). We hypothesised that genetic variations leading to changes within this network of AAs could influence the VP1-CD300lf interaction and viral persistence.

Bioinformatic analysis of the VP1-receptor interface highlighted variation in just a single AA that correlates with persistent MNV strains. To confirm this AA is important for receptor interactions we conducted *in vitro* evolution experiments on suspension or adherent grown cells. Passage through suspension cells resulted in the selection of hydrophobic residues at this position co-incidental with a 1.5-fold increase in viral titre. In contrast, small polar residues were maintained at this position during passage on adherent cells. Furthermore, infectivity assays with infectious clones suggest that hydrophobic residues favour infection of suspension cells over adherent cells. Work is ongoing to understand the importance of this AA on viral infectivity and persistence.

## P241

# Control Of Hepatitis E Virus Polyprotein Processing By Cellular Proteases.

Danielle M. Pierce, Frazer J.T. Buchanan, Abigail Cox, Nicola J. Stonehouse, Morgan R. Herod
University Of Leeds, Leeds, United Kingdom

## Abstract

Hepatitis E Virus (HEV) is one of the leading causes of acute viral hepatitis, with ~20 million HEV infections worldwide per annum, and mortality rates up to 25% in pregnant women. However, many aspects of the biology of the virus are poorly understood. HEV has a positive-sense single-stranded RNA genome. ORF1 encodes the non-structural polyprotein required for viral RNA replication. This polyprotein (sometimes termed pORF1) is predicted to contain seven domains based on sequence homology to related viruses and it is hypothesised that this polyprotein must undergo proteolysis to generate functional protein units. However, it is unknown if the pORF1 polyprotein undergoes full proteolysis, the potential locations of any cleavage boundaries and whether a viral or host cell protease is responsible.

We have adapted our *in vitro*-based proteolysis assays to investigate cleavage of HEV pORF1. In comparison to related RNA viruses, our data suggest that pORF1 has no auto-catalytic activity. Previous studies have shown that the liver-produced protease, thrombin, is essential for replication. In the presence of thrombin, we have shown that the ORF1 polyprotein undergoes specific proteolysis to produce eight distinct protein products. Combining bioinformatics with pORF1 truncations/mutagenesis, we have located the position of the pORF1 thrombin cleavage sites. Interestingly, these cleavage sites correspond to the junctions between the predicted pORF1 protein domains. Our data suggests that thrombin is an important cellular protease for controlling pORF1 proteolysis. Work is ongoing to understand the importance of each thrombin cleavage site in viral replication.

## P242

# Identification of Residues within Flavivirus Non-Structural Protein Associated with Mosquito Transmission

Boglarka Vamos[1], Yin Cheung[1], Kevin Maringer[2,1]
[1]University of Surrey, Guildford, United Kingdom. [2]The Pirbright Institute, Pirbright, United Kingdom

## Abstract

Flavivirus non-structural protein 1 (NS1) is present at high levels in patient blood and has been previously implicated in increasing virus acquisition by mosquito vectors, which may facilitate increased transmission. Therefore, flaviviruses transmitted by *Aedes aegypti* mosquitoes were hypothesised to have similarities within NS1, and potentially other NS proteins, that may correlate with mosquito-borne (rather than tick-borne or no vector) transmission. Representative flavivirus amino acid sequences were downloaded from the NCBI Protein database, aligned using viprbrc.org and then manually analysed to identify residues correlating with arthropod vector tropism. All available sequences for individual clinically important flaviviruses were then analysed on viprbrc.org to determine sequence conservation within viral species for those residues correlating with vector tropism. Overall, we found five residues in NS1 that were highly correlated with transmission by mosquitoes, had functionally relevant differences in amino acid side chain properties between mosquito- and tick-borne viruses and had conservation within viral species. Additionally, a further nine residues in NS3 and four residues in NS4A were identified using the same methodology. Importantly, for NS1, for which more data is available regarding its role in influencing transmission, the residues we identified have not been implicated in increased transmission thus far. Hence, future work would aim to test the impact of mutating these NS1 residues to elucidate whether they modulate NS1 secretion into the blood stream, virus replication and/or transmission. Gaining an understanding of the molecular determinants underpinning vector tropism could be useful in the creation of transmission-incompetent vectors.

## P243

# Recombination, and Then What? How Zika Virus Hybrids Improve Their Fitness

Michaela Dermendjieva[1], Claire Donald[2], Alain Kohl[2], David Evans[1]
[1]University of St Andrews, St Andrews, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom

## Abstract

Zika virus (ZIKV) is an emerging flavivirus primarily transmitted through the bite of *Aedes* mosquitoes. It spread explosively around the tropics in the 21st century, and still presents a threat to human health. There is a need to better understand the drivers of emergence of ZIKV and likely consequences of its future evolution. RNA recombination is a process of genetic exchange which occurs in positive-sense RNA viruses and contributes to virus evolution. We have developed a recombinant-specific PCR assay for detection of ZIKV recombinants in mammalian and insect cell co-infections. Using this system, we demonstrated the ability of distinct geographic and genetic isolates to recombine and tested their viability in tissue culture. Initial ZIKV recombinants produce plaques with a significantly smaller diameter than either parental strain. Recombinant large-plaque variants were isolated through serial passage and plaque purification, and the genome analysed for adaptive mutations. A single nucleotide coding mutation in NS1 was detected in several independent large-plaque variants. The role of this mutation in virus replication was investigated through reverse genetics to assess whether it gave the recombinant a replicative advantage. Understanding the genetic changes that arise during, and following recombination is important in the context of virus evolution. It is particularly relevant for ZIKV, as co-circulation of the African and Asian strains of ZIKV in nature has already occurred in parts of Latin America and Africa. Current and future studies will focus on the phenotype of recombinant ZIKV, their replication in mammalian and insect cell lines, and in mosquitoes.

## P244

# The origin of H9N2 avian influenza virus internal genes contributes to the fitness of 2013 China origin H7N9

Joe James[1], Sushant Bhat[2], Sarah Walsh[1], Jean-Remy Sadeyen[2], Pengxiang Chang[2], Sahar Mahmood[1], Holly Everest[2], Benjamin Mollett[1], Marek Slomka[1], Sharon Brookes[1], Munir Iqbal[3]
[1]Animal and Plant Health Agency, Weybridge, United Kingdom. [2]The Pirbright Institute, Woking, United Kingdom. [3]The Pirbight Institute, Woking, United Kingdom

## Abstract

In recent years, Chinese-origin H9N2 viruses have donated a cassette of internal genes, generating several notable zoonotic avian influenza viruses including H7N9. We investigated whether reassortment with genetically different H9N2 viruses, from other regions, could produce viable viruses with altered biological properties in chickens. Using reverse genetics we generated a panel of reassortant viruses, each containing the HA and NA from H7N9 (A/Anhui/1/13), plus the remaining six internal genes from three different H9N2 viruses representative of dominant H9N2 lineages in Asia and the Middle East. H9N2 internal gene cassettes from two G1-like H9N2 viruses showed attenuation in infected chickens, with reduced shedding profiles and impaired transmission to naive chickens. However, the possession of a BJ94-like H9N2 internal genes resulted in efficient transmission and significantly elevated cloacal shedding compared to the *wt* H7N9 Anhui. Co-infection of chickens with Anhui, and viruses possessing different H9N2 internal gene cassettes, exhibited a limited ability to reassort, yet the propensity for reassortment appeared dependent on the specific H9N2 cassette. Different combinations of H9N2 internal gene cassettes and novel reassortant viruses were identified via co-infection, and possessed different propensities for replication in human cells, suggesting that certain genotypes could pose a zoonotic risk. In summary, the lineage of H9N2 internal gene cassettes alters the infectivity and transmissibility in chickens and replication in human cells. Therefore the emergence of novel epizootic and zoonotic viruses by reassortment with indigenous poultry-adapted H9N2 viruses is more likely to occur with certain H9N2 genotypes.

## P245

# The mutational variety of the live-attenuated influenza vaccine proteome

Megan McConnell[1], Léa Meyer[2], Edward Hutchinson[2]
[1]University of Glasgow, Glasgow, United Kingdom. [2]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

## Abstract

Influenza viruses evolve rapidly, and for this reason the influenza vaccine needs to be updated every year. It is therefore important to identify where new mutations could be tolerated. To assess this, we asked which mutations could be identified in the proteins that had passed quality checks by being correctly folded, transported and assembled into influenza virus particles. We re-analysed mass spectrometry proteomics data obtained from the virus particles of genetically well-defined vaccine strains and identified point mutations within viral proteins. Point mutations were tolerated in virus particles at appreciable frequencies in proteins of both influenza A and B viruses, including HA, NA, M1, NP, NS1, PA and PB2. Structural analyses were used to assess the likely impact of this protein diversity on the molecular biology of the virus. As would be expected, mutations that were tolerated the virus particle generally occurred at sites that would not be expected to perturb protein function. We suggest that using proteomics to identify sites in viral proteins that either can or cannot tolerate mutations could inform influenza vaccine development by highlighting areas that have the potential for antigenic drift.

## P246

# Dengue Virus Non-Structural Protein 1 Disrupts the Interaction Between Endothelial Cells and Pericytes Causing Hyperpermeability

Yin Cheung[1], Davide Maselli[1], Valeria Mastrullo[1], Paolo Madeddu[2], Kevin Maringer[1], Paola Campagnolo[1]
[1]University of Surrey, Surrey, United Kingdom. [2]Bristol Heart Institute, Bristol, United Kingdom

## Abstract

High levels of the secreted non-structural protein 1 (NS1) have been shown to contribute to dengue haemorrhagic fever (DHF), via multiple mechanisms affecting endothelial cells. However, previous studies have not considered the potential role of pericytes, which are perivascular cells wrapped around the endothelial monolayer that tightly regulate endothelial barrier integrity. Dysfunction of pericytes contributes to other haemorrhagic pathologies, such as diabetes retinopathy. This study aims to investigate whether the interactions between endothelial cells and pericytes are affected by NS1. Transepithelial electrical resistance (TEER), an in vitro measure of permeability, was strongly affected by the addition of NS1 and the effect was markedly increased when endothelial cells were grown in co-culture with pericytes, as compared to endothelial cells cultured alone. This suggests that NS1 impacts endothelial cell-pericyte interactions, amplifying its direct effect on endothelial cells alone. In a 3D angiogenesis assay, the co-culture of endothelial cells and pericytes increases the angiogenic potential of endothelial cells. Upon NS1 treatment this effect was blunted, as measured by the reduction of the width of vascular tubules formed. Importantly, these effects were observed at lower and more patient-relevant concentrations of NS1 than in the absence of pericytes. The observed results were not dependent upon cell viability, as AlamarBlue assay showed no significant change in pericyte or endothelial cell proliferation upon NS1 treatment. We conclude pericytes play a significant role maintaining endothelial monolayer integrity that is disrupted by NS1 during dengue-associated vascular hyperpermeability. Unravelling this pathway could provide new targets for dengue diagnosis and treatment.

## P247

# Delineating the interaction between Influenza A viral protein Matrix protein 1 and host factor Transportin-1

Carl Winn[1], Carolyn Nicolson[1], Othmar Engelhardt[1], Jason Long[1], Yohei Yamauchi[2]
[1]NIBSC, Potters Bar, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

## Abstract

Influenza A virus (IAV) is the cause of seasonal epidemic and pandemic influenza disease. Currently, influenza vaccines, such as the inactivated and live attenuated influenza vaccines (LAIVs) offer the best protection against serious influenza disease. However, current influenza vaccines have limitations such as variable vaccine effectiveness. Further understanding the IAV entry and uncoating process may lead to the identification of interactions that may be exploited to generate attenuated viruses. A recent study identified Transportin-1 (TNPO1) as an IAV uncoating factor that interacts with the viral protein Matrix protein 1 (M1) upon exit of the late endosome and entry into the cytosol. Depletion of TNPO1 resulted in reduced IAV infection and replication. Furthermore, substitution of an M1 residue thought to be involved in the TNPO1 interaction resulted in the mutant virus reaching 2% infectivity compared to the wild-type virus. Therefore, this interaction presents a potential target for virus attenuation. We hypothesise that substitution of key residues identified in past studies and through bioinformatic analysis, such as protein-protein docking, will disrupt the interaction between M1 and TNPO1, which can subsequently be measured *in situ* using the highly sensitive proximity ligation assay. Delineation of the interaction between M1 and TNPO1 may open the possibility of the development of targeted antivirals and novel methods of virus attenuation.

## P248

# Phenotypic analysis of epidemic Zika virus isolates.

Alanna Gallagher[1], Goedele N. Maertens[1], Efstathios S. Giotis*[2,1], Carolina Herrera*[1], Michael A. Skinner*[1]
[1]Imperial College London, London, United Kingdom. [2]University of Essex, Colchester, United Kingdom

## Abstract

Zika virus (ZIKV) is a single stranded, positive sense RNA, flavivirus. It is transmitted mainly by mosquitos but can also be transmitted through sexual intercourse; vertically from mother to child; and via contaminated blood. ZIKV isolates from the 2013-14 French Polynesian epidemic and the 2015-16 South America/Caribbean epidemic are closely related sharing approximately 99% nucleotide identity, with minimal non-synonymous mutations. Yet, phenotypic differences between these isolates have been reported for infections in *Ifnar1*$^{-/-}$ mice and wild-type C57BL/6J developing mice. In this study, we initially established an *ex vivo* model with human colorectal tissue explants to compare the phenotypes associated with infection by the ZIKV isolates H/PF/2013 (HPF; French Polynesia) and PRVABC56 (PR; Puerto Rico). We observed that PR replicated quicker and yielded higher titres then HPF and both strains induced distinct inflammatory cytokine profiles. These isolates differ by only 4 amino acids and by a single nucleotide in the highly structured 3'UTR, offering an opportunity to associate phenotypic differences with genetic determinants. To dissect the phenotypic impact of these genetic differences, a previously published reverse genetics system based on a Brazilian ZIKV isolate (Paraiba_01/2015) is being employed to create ZIKV chimeras, to be tested *ex vivo* and *in vitro* by comparing viral yield, replication kinetics, cytokine levels (*ex vivo* only) and host gene expression.

## P249

# A possible role of nonstructural protein 1-specific immunoglobulin G antibody enzyme-linked immunosorbent assays in post-dengue-vaccine era

Jedhan U. Galula[1], Raul V. Destura[2], Roland Remenyi[3], Day-Yu Chao[1]
[1]National Chung Hsing University, Taichung, Taiwan. [2]University of the Philippines, Manila, Philippines.
[3]The Medical City, Manila, Philippines

## Abstract

Infection with dengue virus (DENV), composed of four antigenically distinct serotypes (DENV1-4), remains a global health concern. A commercially available vaccine, Dengvaxia®, is only recommended for individuals in dengue-endemic countries and with laboratory-confirmed exposure. Enzyme-linked immunosorbent assays (ELISAs), which detect antibodies targeting DENV envelope (E) proteins, represent the front-line serological test for presumptive dengue diagnosis. Antibodies targeting the nonstructural protein 1 (NS1) may also serve as surrogate detection markers to distinguish immunity due to vaccination from natural infection. Whereas the literature on anti-E assays is more extensive, fewer studies have evaluated the accuracy of detecting anti-NS1 antibodies. Here, we evaluated the diagnostic performances of two anti-dengue NS1 antibody-based ELISA methods, immunoglobulin G antibody-capture ELISA (GAC-ELISA) and indirect NS1 IgG ELISA, and compared results with a virus-like particle (VLP)-based GAC-ELISA. NS1-based methods had comparable accuracies as E-based VLP GAC-ELISA, as demonstrated by the receiver operating characteristic (ROC) curve analyses with high area-under-the-curve (AUC) values of more than 0.90. Although sensitivity in detecting anti-NS1 IgM was poor, indirect NS1 ELISA showed similar sensitivity as NS1 GAC-ELISA in detecting anti-NS1 IgG. Both NS1 GAC- and indirect IgG ELISAs showed similar limits of detection (~1 to 2 ng/ml). Lastly, combining results from two or more tests as a composite reference standard can determine the DENV serostatus with a specificity reaching 100%. In conclusion, our study shows comparable accuracies in determining serostatus using indirect NS1 IgG ELISA and NS1/VLP GAC-ELISAs. In the future, this approach could assist clinicians to effectively assess vaccine eligibility.

## P250

# Changes in CD4+CD25+TGFβ+ Treg and CD4/CD8-double positive cell populations in chickens of different age groups following IBDV infection that could contribute to immunosuppression

Salik Nazki[1], Vishwanatha Reddy[1], Nitin Kamble[1], Shahriar Behboudi[1,2], Holly Shelton[1], Andrew Broadbent[1,3]
[1]The Pirbright Institute, Woking, United Kingdom. [2]Department of Pathology and Infectious Disease, School of Veterinary Medicine, University of Surrey, Guildford, United Kingdom. [3]Department of Animal and Avian Sciences, University of Maryland, College Park, USA

## Abstract

Infectious Bursal Disease Virus (IBDV) causes immunosuppression in chickens. The virus destroys B cells, likely responsible for the suppression of humoral immunity, however, the mechanism of suppression of cellular immunity is poorly understood. To address this, chickens of two days or two weeks of age were inoculated with vaccine and field strains and the immune-cell populations quantified and compared to mock. Briefly, three groups of 24 birds in each age group were intranasally inoculated with 100µl of PBS, vaccine strain (228E) and classic field strain (F52-70) ($10^5$ $TCID_{50}$ / bird), and six birds from each group were culled at 7, 14, 28, and 35 days-post-infection. No clinical signs were observed in birds inoculated with PBS or vaccine, however, older birds infected with the field strain showed clinical signs and 5 reached humane endpoints. The vaccine and field strains led to atrophy of the Bursa of Fabricius (BF), causing a significant reduction of $Bu1^+$-B cells, yet a significant increase in $CD4^+$ and $CD8^+$-T cells. Interestingly, there was also a significant increase in CD4+CD25+ TGFβ+ and $CD4^-$/CD8-double positive cells in the BF. We speculate that the CD4+CD25+ TGFβ+ cells are Tregs, and there is evidence in the literature that $CD4^-$/CD8-double positive cells can also produce IL-10 and have a suppressive phenotype. In summary, even though birds inoculated with the 228E strain did not show clinical signs, the birds were almost as immunosuppressed as F52-70-infected birds, and we hypothesise that Tregs and CD4/CD8 double-positive cells contribute to IBDV-mediated suppression of cellular immunity.

## P251

# Addressing the emerging burden and threat from Influenza B viruses via the development of Hemagglutinin (HA) and Neuraminidase (NA) pseudotype virus panels

Kelly da Costa[1], Joanne Marie Del Rossario[1], Francesca Ferrara[2], George Carnell[3], Jonathan Heeney[3], Nigel Temperton[1]
[1]Medway School of Pharmacy, Chatham, Kent, United Kingdom. [2]St Jude Children's Research Hospital, Memphis, USA. [3]University of Cambridge, Cambridge, United Kingdom

### Abstract

Influenza B virus (IBV) is responsible for 25% of all seasonal influenza infections, which until recently has been underestimated. IBV has not caused pandemics in the past and has therefore not been studied as extensively as Influenza A (IAV). Furthermore, traditional serological assays for influenza A surveillance such as hemagglutination inhibition (HI) and Single Radial Hemolysis (SRH) have been shown to not be as sensitive to IBV responses.

To address this, we have developed two IBV pseudotype virus (PV) panels: a hemagglutinin (HA) panel, expressing IBV HA, and a neuraminidase (NA) panel expressing IBV NA, from both Victoria-like and Yamagata-like lineages. We demonstrate optimisation of production methods for each panel to maximise individual PV production titres. Interestingly, when HA is co-expressed with IBV NA, PV production titres are increased, but background responses when performing enzyme-linked lectin assay (ELLA) increase, in contrast to what we observe with IAV PV.

Both IBV HA and NA pseudotype panels have been employed to great effect as substitutes for wildtype viruses in microneutralisation (pMN) and ELLA assays. This system was used successfully to assess effectiveness of drug treatment against IBV, neutralising capacity of monoclonal antibodies (mAbs), serum response to vaccination in both humans and mice, enhancement of pre-existing IBV immunity in individuals, and the ability to evaluate cross-subtype neutralising responses to both HA and NA. We propose that these panels could be employed to enhance IBV surveillance, vaccination and therapeutic interventions in the future.

## P252

# Therapeutic potential and function of the conserved s2m element in RNA viruses

Valeria Lulla[1], Michal Wandel[2], Katarzyna Bandyra[3], Rachel Ulferts[4], Mary Wu[4], Tom Dendooven[3], Xiaofei Yang[5], Nicole Doyle[6], Stephanie Oerum[7], Rupert Beale[4], Sara O'Rourke[8], Felix Randow[2], Helena Maier[6], William Scott[8], Yiliang Ding[5], Andrew Firth[1], Kotryna Bloznelyte[3], Ben Luisi[3]
[1]Department of Pathology, University of Cambridge, Cambridge, United Kingdom. [2]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom. [3]Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom. [4]The Francis Crick Institute, London, United Kingdom. [5]Department of Cell and Developmental Biology, John Innes Centre, Norwich Research Park, Norwich, United Kingdom. [6]Pirbright Institute, Pirbright, United Kingdom. [7]CNRS-Université Paris Diderot, Institut de Biologie Physico-Chimique, Paris, France. [8]University of California at Santa Cruz, Santa Cruz, USA

## Abstract

RNA structural elements occur in numerous single stranded (+)sense RNA viruses. The stem-loop 2 motif (s2m) is one such element with an unusually high degree of sequence conservation, being found in the 3' UTR in the genomes of many astroviruses, some picornaviruses and noroviruses, and a variety of coronaviruses, including SARS-CoV and SARS-CoV-2. The evolutionary conservation implicates a key role of s2m in the viral infection cycle. We explore this element from functional and therapeutic perspectives using human astrovirus, SARS-CoV-2 and various reporter systems. Our findings indicate that the s2m element plays an important role in the virus life cycle and can be targeted by antisense oligonucleotides (ASOs) that initiate pairing in exposed loops and trigger efficient sequence-specific RNA cleavage in reporter assays. The anti-viral potential of this conserved element represents a promising start for further research into targeting conserved elements in single-stranded (+)sense RNA viruses.

## P253

# Experimental Virus Challenge in Calves to Identify Genetic Markers of Bovine Respiratory Syncytial Virus Infection.

Dayle Johnston[1], Bernadette Earley[1], Matthew McCabe[1], Gordon Blackshields[1], Ken Lemon[2], Catherine Duffy[2], Michael McMenamy[2], Rachael Bell[2], Hannah Turkington[2], Jaewoo Kim[3], Jeremy Taylor[3], Sara Louise Cosby[2], Sinead Waters[1]
[1]Teagasc, Dunsany, Ireland. [2]Agri-Food and Biosciences Institute, Belfast, United Kingdom. [3]University of Missouri, Columbia, USA

## Abstract

Bovine respiratory disease is one of the most economically important diseases for the Agri-Food industry globally being the main cause of cattle mortality. The initial insult is usually due to viral infection, with bovine respiratory syncytial virus (BRSV) a common cause. Viral infections predispose calves to secondary bacterial infections, similar to human RSV. Experimental infection allows identification of genetic markers of susceptibility/resistance and informs vaccine design/therapy for both bovine and human RSV. Holstein-Friesian calves were challenged with BRSV or mock challenged. Respiratory virus loads and clinical/immunological parameters were examined. Calves were euthanised (day 7) and bronchial lymph nodes/blood harvested. RNA was extracted and RNA-Seq/miRNA libraries prepared/sequenced. Sequenced reads were adapter trimmed/quality assessed and aligned to the bovine genome (UMD 3.1). EdgeR was used for differential expression analysis and Targetscan to identify target genes for differentially expressed (DE) miRNAs. Pathway and gene ontology analyses of target genes were carried out using Ingenuity Pathway Analysis Platform. Multidimensional scaling analysis showed clear separation between BRSV challenged and control calves with 934 DE genes in lymph nodes with similar findings in blood. Of the DE predicted target genes, 455 had fold-changes in an inverse direction to that of their DE miRNAs, associated with 8 enriched pathways. Immunological/pathological results were consistent with mRNA/miRNA findings. This study identified mRNA biomarkers/molecular pathways of BRSV infection, being the first to do so in circulating whole blood samples, delivering a novel development for diagnostics and assessment of vaccine efficacy.

## P254

# Development of CRISPR/Cas9-based Novel Vaccines against Poultry Viruses

Julianne Vilela, Muhammad Munir
Division of Biomedical and Life Sciences, Faculty of Health and Medicine, The Lancaster University, Lancaster, United Kingdom

## Abstract

Vaccines remain the primary means of disease prevention through immunisation schemes in the poultry sector. Novel approaches in vaccine development, such as reverse genetic systems and genome editing technologies (i.e. CRISPR/Cas9), are currently being utilized to overcome challenges in establishing an immunogenic platform that is safe and capable of inducing long-term immunity. The CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats associated protein nuclease 9) technology offers an effective, fast and simple novel approach to edit genomes for the development of viral vectors against poultry diseases such as Newcastle disease (ND), avian influenza (AI) and infectious bronchitis (IB). In this study, we demonstrate the application of CRISPR/Cas9-based genome editing to generate recombinant viral vectors that express the F gene of NDV. Validation of gene integration, protein expression, and insert stability was carried out by PCR, immunostaining and Western blot analysis, respectively. This approach offers an efficient platform for the generation of multivalent recombinant vaccines that can provide simultaneous protection against major poultry diseases.

## P255

# Evolutionary Conservation of the potential N6-methyladenosine ($m^6A$) sites Among Influenza A Viruses

Mahmoud Bayoumi, Muhammad Munir
Division of Biomedical and Life Sciences, Lancaster University, Lancaster, United Kingdom

## Abstract

The addition of a methyl group to the N6-position of adenosine ($m^6A$) is considered the most prevalent internal post-transcriptional modification and is attributed to virus replication and cell biology. Viral epitranscriptome sequencing analysis has revealed that hemagglutinin (HA) mRNA of H1N1 carry eight $m^6A$ sites which are primarily enriched in 5'-DRACH-3' sequence motif. Herein, a large-scale comparative analysis was conducted to investigate the conservation patterns of the DRACH motifs that corresponding to the reference $m^6A$ sites among influenza A viruses. A total of 70,030 complete HA sequences that comprise all known HA subtypes (H1-18) collected over several years, countries, and affected host species were analysed on both mRNA and vRNA strands. The bioinformatic analysis revealed the highest degree of DRACHs conservation among all H1 sequences that clustered largely in the middle and in the vicinity to 3' end with at least four DRACH motifs were conserved in all mRNA sequences. The major HA-containing subtypes displayed a modest DRACH motif conservation located either in the middle region of HA transcript (H3) or at the 3' end (H5) or were distributed across the length of HA sequence (H9). The lowest conservation was demonstrated in HA subtypes that infect mostly the wild avian species and bats. Interestingly, the total number and the conserved DRACH motifs in vRNA were found to be much lower than those observed in mRNA. Collectively, the identification of putative $m^6A$ topology provides a foundation for the future intervention of influenza infection, replication, and pathobiology in susceptible hosts.

## P256

# INFLUENZA A NEURAMINIDASE PSEUDOTYPES (N1-N9) AS TOOLS FOR PANDEMIC PREPAREDNESS AND IMPROVED INFLUENZA VACCINES

Joanne Marie Del Rosario[1], Kelly da Costa[1], Fabrizio Biuso[1], George Carnell[2], Matteo Ferrari[2], Jonathan Heeney[2], Nigel Temperton[1]
[1]University of Kent, Chatham, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom

## Abstract

Influenza contains a lipid membrane studded with two membrane glycoproteins, hemagglutinin (HA) and neuraminidase (NA). It has been established that antibodies to NA protect against influenza infection during seasonal and pandemic outbreaks, independent of anti-HA response. Inhibition of NA activity is also a target of licensed small-molecule drugs (NA inhibitors) that are routinely used for influenza treatment. These known prophylactic and therapeutic effects validate NA as an antigen, nonetheless, NA has been largely ignored in favor of HA in the formulation of current influenza vaccines.

To better understand how neuraminidase contributes to protection against influenza and to investigate its breadth and possible cross-neutralizing activity, we have produced lentiviral vectors pseudotyped with an avian H11 head and the NA (N1-N9) of all influenza A NA subtypes, covering current and emerging zoonotic threats. These NA viral pseudotypes (PV) provide stable NA activity and can be utilized as target antigens for *in vitro* assays to measure monoclonal antibody and antiviral drug activity, and vaccination efficacy. Employing these NA PV, we have developed an enzyme-linked lectin assay (ELLA) that demonstrates the independent contribution of NA to protection against influenza. We have optimized our ELLA for routine serology to measure neuraminidase inhibition (NI) titers following vaccination with various influenza antigens.

Our studies may lead to establishing the protective NA titer that contributes to NA-based immunity. This will aid in the design of better, longer lasting, and more broadly protective vaccines that can be employed together with HA assays as a pre-pandemic response approach.

## P257

# Evaluating the diversity and reassortment potential of infectious bursal disease virus (IBDV) strains in the UK

VISHWANATHA REDDY[1], Carlo Bianco[2], Alex Schock[2], Salik Nazki[1], David Welchman[3], Yongxiu Yao[1], Andrew Broadbent[1,4]
[1]THE PIRBRIGHT INSTITUTE, WOKING, United Kingdom. [2]Animal and Plant Health Agency (APHA), Lasswade, United Kingdom. [3]Animal and Plant Health Agency (APHA), Winchester, United Kingdom. [4]University of Maryland, Maryland, United Kingdom

## Abstract

Infectious bursal disease virus (IBDV) is ranked among the top-five infectious problems of chickens worldwide. IBDV has a bi-segmented double-stranded RNA genome comprised of segments A and B. Classical (c), vaccine, and very virulent (vv) strains can be distinguished by sequencing the VP2 capsid gene of segment A and the VP1 polymerase gene of segment B, however, reassortment complicates the epidemiology worldwide. In the UK, there has not been a molecular epidemiological survey of IBDV for several years. Hence, we sequenced regions of the VP2 and VP1 of 10 field samples obtained from seven UK broiler farms in 2020 that were vaccinated against IBDV 7-37 days previously. IBD was suspected on clinical grounds. The histological lesions were consistent with IBD in 3/7 farms, while in 4/7 chronic, non-specific atrophy was detected. Phylogenetic analysis revealed that two samples contained segments A and B consistent with vaccine strains, and one sample contained a vv segment A and a c/vaccine segment B, indicating this was a reassortant. Further analysis of chromatogram traces revealed that the remaining 7/10 samples were mixed, suggesting we had co-amplified sequences from both vv and vaccine strains. Following passage *in vitro*, only one of the mixed samples led to a cytopathic effect, suggesting the majority of vaccinated birds were no longer shedding live virus. In summary, we found that vv IBDV field strains circulated in vaccinated flocks and co-amplification of vv and vaccine sequences were common, suggesting that the opportunity for co-infection and reassortment in the UK is high.

## P258

# Engineered anti-viral shRNAs are more effective than lhRNAs in transgenic *Aedes aegypti*

Priscilla Tng[1,2], Leonela Carabajal Paladino[1], Michelle Anderson[1], Vishaal Dhokiya[1], Elizabeth Keen[1], Zach Adelman[3], Rennos Fragkoudis[1,4], Rob Noad[2], Luke Alphey[1]
[1]The Pirbright Institute, Woking, United Kingdom. [2]Royal Veterinary College, Hatfield, United Kingdom. [3]Texas A&M University, College Station, USA. [4]The University of Edinburgh, Edinburgh, United Kingdom

## Abstract

The *Aedes aegypti* mosquito is a major vector of chikungunya virus (CHIKV), which has no licensed vaccine. Engineered mosquitoes expressing long RNA hairpins (lhRNA) or small RNAs against selected arboviruses have been developed to limit virus replication, but their silencing efficiency has not been compared.

We developed lhRNA and short hairpin RNA (shRNA) arrays against CHIKV non-structural protein nsP2. We used a Tet-response element (TRE) and a tTA transactivator to control expression of lhRNAs (TRE-lhRNA) and shRNAs (TRE-shRNA). Constitutive expression in Aag2 cells was assessed with a PUb-tTA driver and midgut specific expression in transgenic mosquitoes was assessed using Carboxypeptidase A (AeCPA)-tTA as driver. *In vitro* interference ability was determined with a CHIKV split replication system, and a synthetic luciferase reporter with mRNA containing the targeted CHIKV sequence (CHIK-FF). *In vivo* interference was tested by inserting the TRE-lhRNA and TRE-shRNA constructs into a AeCPA-tTA line so both constructs expressed from the same locus, and a TRE reporter line that expressed an AmCyan reporter with an N terminal CHIKV fusion (CHIK-AmC).

In Aag2 cells, shRNAs were more effective than lhRNA in silencing both the CHIKV split replication system (99.7%, S.D. ± 0.47 and 72.8%, S.D. ± 9.50, respectively, $P<0.001$) and CHIK-FF (98.8%, S.D. ± 0.71 and 50.9%, S.D. ± 7.92, respectively, $P<0.05$). Similar results were observed in transgenic mosquitoes when comparing AmC expression in the midgut.

This study demonstrates that, in mosquitoes, effectively chosen shRNAs can induce greater interference of the desired viral target than the corresponding lhRNA.

## P259

# Immunisation of cows with Lassa Fever virus glycoproteins elicits potent and broadly neutralising antibodies that extends to other Mammarenavirus species

Theocharis Tsoleridis[1,2], Richard A. Urbanowicz[1,2,3], Joseph G. Chappell[1,2], Helena Müller[4], Sarah Katharina Fehling[4], Emma Bentley[5], Edward Wright[6], David Haig[7,2], Thomas Strecker[4], Jonathan K. Ball[1,2]
[1]School of Life Sciences, University of Nottingham, Nottingham, United Kingdom. [2]Wolfson Centre for Global Virus Infections, University of Nottingham, Nottingham, United Kingdom. [3]Department of Infection Biology and Microbiomes, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [4]Institute for Virology, Philipps University Marburg, Marburg, Germany. [5]Division of Virology, National Institute of Biological Standards and Control, South Mimms, Potters Bar, Herts, United Kingdom. [6]University of Sussex, Brighton, United Kingdom. [7]School of Veterinary Medicine and Science, The University of Nottingham, Nottingham, United Kingdom

## Abstract

Lassa Fever virus (LFV) causes regular outbreaks of haemorrhagic fever in Western Africa, leading to significant morbidity and mortality. Ribavirin therapy is effective, but only if given in the early stages of infection. Therefore, preventative vaccines or more effective treatments, such as monoclonal antibodies, are needed. Antibody-based therapies are proving beneficial in tackling other emerging virus outbreaks, such as Ebolavirus. Vaccine and monoclonal antibody development are complicated by the extensive genetic diversity of LFV. To better understand determinants of potent and broadly neutralising epitopes, as well as to identify novel lead therapeutic antibodies, we performed extended immunisations of cows with mammalian-expressed viral glycoproteins from a single strain of LFV. A cow animal model was chosen because of their ability to express antibodies with extended and highly structured CDR3 regions, which have been implicated in high affinity interaction with, often occluded, virus receptor binding domains. A series of prime and boost immunisations over a period of greater than 52 weeks yielded antibodies that recognised and neutralised five genetic lineages of LFV. Reactivity and neutralisation also extended to Lymphocytic Choriomeningitis Mammarenavirus. Demonstration of pan-genus neutralisation highlights the therapeutic potential of bovine antibodies and provides molecular insights into cross-neutralising epitopes that might be important for future vaccine design.

## P260

# Temporal changes in influenza virion composition and infectivity over the course of infection.

Seema Jasim, Pippa Harvey, Patrick Shearer, Colin Loney, Edward Hutchinson
University of Glasgow, MRC-Centre for Virus Research, Glasgow, United Kingdom

## Abstract

The virions of influenza A viruses (IAV) are pleomorphic and have a flexible composition. Previously, we used mass spectrometry to characterise the viral and host proteins present in mixed populations of IAV virions produced over multiple cycles of infection. As the proteome of a cell undergoes profound changes during an infection, we hypothesised that the composition of virions shed from a cell would change as infection progressed. To test this, we purified IAV virions and similarly-sized microvesicles shed from cells at different points during synchronised single-cycle infections, and then characterised their proteomes using mass spectrometry and label-free quantitation. We found that throughout infection influenza virions maintained a 'core architecture' of viral proteins in a consistent ratio. However, as infection progressed the amount of the immunosuppressive protein NS1 present in virions increased exponentially. The amount of NS1 shed in microvesicles also increased exponentially between early and late infection. Using confocal microscopy, we confirmed that secreted NS1 was taken up by newly-infected cells. To determine if NS1 shedding correlated with infectivity, we infected cells at equal multiplicities with material shed early or late in infection, and found that the 'late' material caused viral antigen to accumulate more rapidly. We concluded that as infections progress, IAV virions incorporate increasing quantities of NS1 and become more efficient at initiating infection. We propose that these temporal changes in influenza virion composition may help overcome activation of innate immune responses.

## P261

# Rapid sensitive detection and pathotyping of emergent clade 2.3.4.4b avian influenza viruses in Eurasia in UK wild birds

Joe James, Amanda Seekings, Ian Brown, Katie Purchase, Sahar Mahmood, Paul Skinner, Rowena Hansen, Ashley Banyard, Scott Reid
Animal and Plant Health Agency, Weybridge, United Kingdom

## Abstract

Avian influenza viruses (AIVs) can be separated into highly pathogenic (HPAIV) and low pathogencity (LPAIV), based on clinical manifestations in poultry; this pathogenicity correlates with molecular sequence motifs in the haemagglutinin (HA) gene cleavage site (CS). Since 2014, Europe has experienced multiple incursions of subtype H5 HPAIV; including a strain of HPAIV H5N8 resulting in the death of over 300 wild bird in the United Kingdom (UK) since November 2020. Historically, the determination of H5 AIVs in the UK has relied upon the initial detection of an influenza A virus matrix gene-positive sample by real-time reverse transcription polymerase chain reaction (RRT-PCR) followed by subtyping using HA-specific RRT-PCRs, with subsequent pathotyping of positive RRT-PCR samples by Sanger sequencing of the HA gene CS. This algorithm is highly dependable, robust and enables pathotyping of HPAIVs within 24 hours of a positive subtyping H5 RRT-PCR result from the clinical sample. However, a faster methodology was sought to optimise pathotype determination from wild bird clinical samples during the 2020/2021 UK H5N8 epizootic. Here, we describe the successful adaptation and validation of a RRT-PCR that enables pathotype discrimination based on the CS from clinical samples. This assay specifically and reproducibly detects only HPAIV H5 viral RNA with comparable sensitivity to the frontline H5-specific subtyping RRT-PCR. Low pathogenicity AIV H5 RNA and non-AIV RNA were not detected. When run alongside the H5-specific RRT-PCR, the assay significantly reduces time-to-pathotype determination, thereby enhancing the diagnostic workflow whilst reducing costs around defining HPAIV-infected wild birds.

## P262

# TMEM16A as a novel target for the treatment of human respiratory syncytial virus

Hayley Pearson, Jamel Mankouri
University of Leeds, Leeds, United Kingdom

## Abstract

Human respiratory syncytial virus (HRSV) is a common cause of respiratory tract infections (RTIs) globally. Of those infected, 25%–40% aged ≤1 year develop severe lower RTIs leading to pneumonia and bronchiolitis, with ~10% requiring hospitalisation. There is no HRSV vaccine and current clinically approved treatments are only moderately effective. New and more effective anti-HRSV strategies are urgently required.

It is now established that viruses require cellular ion channels to infect cells. Here, we demonstrate the requirement for TMEM16A/ANO1, a calcium-activated chloride channel for HRSV infection. Time-of-addition assays revealed that specific TMEM16A blockers inhibit HRSV at a postentry stage, showing activity as a postexposure prophylaxis. Influenza virus was similarly inhibited by the TMEM16A-specific blocker T16Ainh-A01.

These findings reveal TMEM16A as an exciting target for future host-directed antiviral therapeutics. We are now investigating the role of this channel in other RNA virus infections and the mechanism(s) underpinning its requirement by HRSV.

## P263

# Analysis of the role of NS5A domain I in hepatitis C virus genome replication and assembly.

Shucheng Chen, Mark Harris
University of Leeds, Leeds, United Kingdom

## Abstract

Hepatitis C virus (HCV) is an enveloped virus with a positive-sense, single-stranded RNA of approximately 9.6 kb. It is a member of the genus *Hepacivirus* of the *Flaviviridae* family. The genome contains a single large open reading frame encoding a 3000-residue polyprotein. The non-structural 5A protein (NS5A) is highly phosphorylated and is comprised of three domains (I, II and III).

Previously, we demonstrated that domain I of NS5A in genotypes 2a (JFH1) played a role in virus assembly, exemplified by the phenotype of alanine substitutions of two residues (V67 and P145) (Yin *et al*, 2018) which were not required for RNA replication. In this study we extended this analysis to demonstrate that 5 additional residues, surface exposed and proximal to either V67 or P145, exhibited the same phenotype.

In parallel, to investigate the mechanism underpinning this role of domain I we assessed the involvement of cyclophilin A (CypA), a cellular peptidyl-prolyl isomerase required for HCV RNA replication, which can be inhibited by cyclosporin A (CsA). Intriguingly, the 7 mutants exhibited significant differences in sensitivity to CsA treatment, suggesting that domain I might interact with CypA. Using shRNA expressing lentiviruses, we also demonstrate that in Huh7 CypA knockdown cells both wildtype and all mutants were dependent upon CypA for replication. However, in corresponding Huh7.5 CypA-knockdown cells replication was restored for wildtype and a subset of the mutants. These data suggest a complex interplay between NS5A domain I, CypA and other as yet unidentified cellular factors which is the subject of ongoing studies.

## P264

# Development of reporter cell lines for detection of infectious Lassa virus

Christopher Burton
Public Health England, Salisbury, United Kingdom. Oxford Brooks University, Oxford, United Kingdom

## Abstract

Lassa virus (LASV) is the causative agent of Lassa fever, a haemorrhagic disease endemic in West Africa. LASV is highly pathogenic and classified as a Hazard Group 4 pathogen. The scale of seasonal outbreaks has increased dramatically since 2017 with 2019 likely to surpass previous years. This combined with its epidemic nature and social and economic impact LASV has been listed as a priority disease for developing medical countermeasures by the WHO.

Molecular testing is largely used for the diagnosis of LASV because of specificity, speed and ability to be performed in developing countries. LASV has high levels of genetic diversity between strains, increasing the potential of strains evading molecular detection. Virus isolation remains an important tool for diagnosis, as it demonstrates the presence of infectious virus and is less likely to be affected by strain diversity.

Virus isolation requires specialist Containment Level 4 facilities, is labour intensive and can take several weeks. A new LASV infectivity assay which could decrease levels of interpretation, give a quantifiable readout and enable faster detection would be hugely beneficial.

This work aims to produce a stable cell line for the detection of infectious LASV. Specific LASV regulatory sequences along with fluorescent and bioluminescent reporters will be stably integrated into the cell, which will be activated upon infection producing a measurable response. This will enable faster confirmation of infectious virus when compared with current infectivity assays and have further research applications.

## P266

# Determinants of lectin-mediated enhancement of Ebolavirus entry

Richard Urbanowicz[1,2,3], Holly Bamber[1,2], Monika Pathak[1,2,4], Jayasree Dey[1,2], Barnabas King[1,2,5], Jonathan Ball[1,2], Alexander Tarr[1,2]
[1]School of Life Sciences, University of Nottingham, Nottingham, United Kingdom. [2]Wolfson Centre for Global Virus Infections, University of Nottingham, Nottingham, United Kingdom. [3]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [4]School of Pharmacy, University of Nottingham, Nottingham, United Kingdom. [5]Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom

### Abstract

Historically Ebolavirus outbreaks have been sporadic and relatively self-contained, but as globalisation increases, and an ever-expanding human population, our interactions with these pathogens are likely to increase. Whilst virus evolution will play a major factor in outbreak severity it is widely accepted that host factors can also have a significant impact both on susceptibility to infection and also disease severity. Human lectins (MBL and M-ficolin) have been shown to enhance Ebolavirus infection, but the exact mechanism is unknown. It has been proposed that the lectin can act as an intermediate receptor or, by associating with the glycoprotein, alter the inherent infectivity of the virus particle.

Here we selected a panel of plant-derived lectins with the ability to recognise different carbohydrate structures to interrogate the mechanisms of lectin-mediated enhancement. Using an established pseudotype infection assay and the lectins *Wisteria floribunda* agglutin (WFA), Soybean agglutin (SBA) and *Galanthus nivalis* agglutin (GNA) we demonstrate that the enhancement is cell-line independent and that disaccharide-specific lectins (WFA) interact directly with the glycoprotein to enhance entry in an NPC1-dependent manner. This enhancement is independent of lectin interactions with the $GP_{1,2}$ mucin-like domain. Lectin-mediated enhancement did not affect neutralisation sensitivity of the C15 Makona variant to the potent anti-EBOV nAb KZ52

In conclusion we show that the WFA-enhanced infectivity of Ebolavirus is due to its specific disaccharide recognition in the glycoprotein and is not due it acting as an intermediate receptor.

## P268

# Virus damage or host response? Elucidating mechanisms of pathogenesis in bluetongue virus-infected sheep.

Kerry Newbrook[1], Karen Chong[2], Marc Guimera Busquets[1], Lyndsay Cooke[1], Aimee Fisher[1], Amanda Corla[1], Martin Ashby[1], Matt Tully[1], John Flannery[1], Christopher Sanders[1], Jessica Stokes[1], Beatriz Sanz-Bernardo[1], Simon Carpenter[1], Katy Moffat[1], Karin Darpel[1]
[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Surrey, Guidford, United Kingdom

## Abstract

Bluetongue is a non-contagious, haemorrhagic disease of ruminants caused by Bluetongue virus (BTV). BTV is an arthropod-borne Orbivirus (family: Reoviridae) transmitted to its mammalian host by infected *Culicoides* biting midges. Although T lymphocytes become productively infected with BTV, roles of specific T cell subsets in BTV pathogenesis, host antibody responses and transmission have yet to be elucidated.

Sheep were intravenously administered specific monoclonal antibodies to deplete different T lymphocyte subsets. Using a natural *in vivo* infection model, *Culicoides sonorensis* (fed blood containing BTV-4 MOR2009/07) were allowed to feed on sheep to establish infection. Viremia was monitored by BTV segment 10 qRT-PCR. Uninfected *C. sonorensis* were blood-fed on sheep to assess onward BTV transmission. T cell depletion and immune cell dynamics in whole blood during infection were monitored by multi-colour flow cytometry. Host inflammatory cytokine and antibody responses were assessed by ELISA.

Significant depletion of each T lymphocyte subset was observed. The natural *in vivo* infection model resulted in BTV-4 transmission to all sheep, demonstrating typical clinical disease. Depletion of specific T cell subsets resulted in altered clinical disease severity, as well as elevated levels of several key inflammatory cytokines and changes in antibody dynamics. A transient lymphopenia occurred around peak viremia, independently of depletion. Interestingly, *C. sonorensis* developed fully transmissible infection from sheep at peak viremia, but not during later stages of infection. Overall, this work highlights clear, distinct roles for specific T cell subsets in BTV pathogenesis.

## P269

# The investigation of virus-driven intrahepatic cholangiocarcinoma through dysregulated hepatic differentiation

Alexandria Kilvington
University of Leeds, Leeds, United Kingdom

### Abstract

Intrahepatic Cholangiocarcinoma (iCCA) is increasing in incidence worldwide and hepatitis C virus (HCV) infection is a known risk factor. Recent studies of HCV associated hepatocellular carcinoma (HCC) have demonstrated that HCV infection induces oncogenic gene expression patterns, resulting from altered epigenetic profiles. Interestingly, these epigenetic profiles were shown to persist post-treatment with direct acting antivirals (DAAs).

The cellular origin of primary liver cancers can vary due to liver cell plasticity. However, the incidence of mixed iCCA-HCC tumour phenotypes and dual iCCA/HCC risk associated with HCV infection led us to hypothesise that the source of virus-driven malignancies may be virus-infected hepatic progenitor cells (HPCs). HPCs have been shown in our laboratory to be susceptible to HCV infection ex vivo and models of hepatic differentiation using an HPC-like cell line; CD24 low Huh7s, reveal disruption caused by HCV infection. Disruption includes the hijacking of the HIPPO signalling pathway and dysregulation of genes including TMEM45B and NRG1, with oncogenic hallmarks persisting following viral cure revealed by RNA-sequencing.

To explore these findings in relation to cholangiocyte-specific differentiation, we are using our own HPC-like cell line along with an established human induced pluripotent stem cell (hiPSC) cholangiocyte differentiation method. With these cell models together, we aim to establish a robust model of HCV-mediated perturbation of cholangiocyte-specific differentiation, in order to identify new treatments that complement DAA therapy to aid in the elimination of malignancy risk.

## P270

# Enhancement of alphavirus replication in mammalian cells at sub-physiological temperatures

Jinchao Guo, Mark Harris
University of Leeds, Leeds, United Kingdom

## Abstract

Chikungunya virus (CHIKV) is a re-emerging *Alphavirus* transmitted by mosquitoes and causing fever, rash and arthralgia. Currently there are no vaccines or drugs against CHIKV, therefore it is important to understand the molecular details of CHIKV replication. Building on our previous studies (Gao et al, 2019) we generated a panel of mutants in a conserved, surface exposed cluster in the nsP3 alphavirus unique domain (AUD), and tested their replication using a subgenomic replicon (SGR) in a variety of mammalian and mosquito cells. We identified three mutants that replicated poorly in mammalian cells but showed no defects in mosquito cells. Further investigation showed that these mutants were temperature-sensitive, rather than species-specific as they showed no defects in mammalian cells at sub-physiological temperatures (28°C). We also observed similar effects using infectious CHIKV as well as a closely related virus: O'Nyong Nyong virus (ONNV).

Intriguingly, this analysis also revealed that the wildtype SGR replicated much more efficiently at sub-physiological temperatures as compared to 37°C. This was not due to impaired interferon responses as this enhancement was also observed in *Vero* cells. Neither was this due to defects in the unfolded protein response as treatment with ISRIB, an inhibitor of global translation attenuation, did not compensate for the replication defects at 37°C for mutants of CHIKV SGR or ONNV. These observations are potentially important when one considers that alphaviruses are transmitted by mosquito bite, such that the first cells to be infected in mammalian host will be at a sub-physiological temperature.

## P271

# Characterising the role of RNA-binding in the restriction of HIV-1 reverse transcription by REAF/RPRD2

Kathryn Jackson-Jones[1], Rebecca Menhua Fu[1], Katie Duckett[1], Alfredo Castello[2], Richard Sloan[1,3]
[1]Infection Medicine, School of Biomedical Sciences, University of Edinburgh, Edinburgh, United Kingdom. [2]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [3]ZJU-UoE Institute, Zhejiang University, Zhejiang, China

## Abstract

The cellular antiviral factor REAF/RPRD2 can potently restrict HIV-1 by inhibiting reverse transcription, however, the underlying molecular mechanism is not yet well characterised. REAF/RPRD2 is present in T-cells and is highly expressed in macrophages and dendritic cells. REAF/RPRD2 is upregulated in response to poly I:C treatment, but it is not type I interferon-inducible, nor is it under positive selection.

Here we show that depletion of REAF/RPRD2 does not influence the ability of THP-1 cells to produce type I interferon in response to transfected nucleic acids. RNA interactome capture screens indicate that REAF/RPRD2 directly binds RNA and we now show that it binds RNA:DNA hybrids in coimmunoprecipitation assays using the RNA:DNA hybrid antibody s9.6. Preliminary data show that overexpression of REAF/RPRD2 leads to a depletion of RNA:DNA hybrids from the nucleus suggesting that REAF/RPRD2 may act to prevent replication of HIV-1 nucleic acids in the nucleus leading to integration in the host genome.

We reason that RNA-binding is important for the antiviral function of REAF/RPRD2 and specifically that REAF/RPRD2 inhibits HIV-1 reverse transcription through interaction with viral RNA. We are now using a non-RNA-binding REAF/RPRD2 mutant to investigate how RNA-binding underpins the ability of REAF/RPRD2 to inhibit HIV infection, specifically the effect on early HIV-1 replication steps including reverse transcription and integration into host chromatin.

Understanding how REAF/RPRD2 can inhibit HIV-1 replication via nucleic acid binding will help improve understanding of innate immune control of HIV-1 and understanding how cells regulate non-self nucleic acids in the nucleus.

## P272

# Investigating the coding capacity of rotaviruses using a newly developed reverse genetics system

Olga Lee[1], Colin Sharp[1], Rute Maria Pinto[2], Samantha Lycett[1], Paul Digard[1], Eleanor Gaunt[1]
[1]The Roslin Institute, The University of Edinburgh, Edinburgh, United Kingdom. [2]MRC – University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

### Abstract

Bovine rotavirus (RV) infection causes severe diarrhoea in young dairy calves. To understand the mechanisms underlying pathogenicity, a reverse genetics (RG) system to allow gene manipulation of RVs is an essential tool. In 2017, Kanai and co-authors (PNAS, 2017) developed a plasmid-only RG system for the simian RV strain SA11. We have developed an analogous RG system for the bovine RF RV strain which we are using to investigate the coding capacity of RVs.

Dogmatically, the 11-segmented dsRNA RV genome encodes 12 proteins, with each segment except segment 11 considered to be monocistronic. We have found using *in vitro* translation and radiolabelling assays that every RV genome segment can produce multiple polypeptide species. Using bioinformatic analyses, we have identified putative alternative translation initiation sites which may correspond with production of some of the secondary protein products. Site directed mutagenesis to remove candidate translation initiation sites has so far led to the identification of two 5'-proximal AUG codons in segment 1 of RV which together explain the production of one secondary band. Work to introduce this mutation into viruses and their downstream characterisation is ongoing. Uncovering the full coding capacity of RVs and the functions of potential accessory proteins will aid our understanding of how RVs interact with their hosts.

## P273

# Sequential Infection of Poultry with Low and then Highly Pathogenic H7N7 Avian Influenza Viruses: Investigating Factors Contributing to Disease Outcome

Alexander Byrne[1], Shannon Leetham[2,3], Joe James[1], Saumya Thomas[1], Caroline Warren[1], Fabian Lean[4], Alejandro Núñez[4], Ashley Banyard[1], Ian Brown[1], Marek Slomka[1], Sharon Brookes[1]
[1]Virology Department, Animal and Plant Health Agency, New Haw, United Kingdom. [2]Virology Department, Animal and Plant Health Agency, New Har, United Kingdom. [3]School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom. [4]Pathology Department, Animal and Plant Health Agency, New Haw, United Kingdom

## Abstract

Factors driving the evolution of high pathogenicity avian influenza viruses (HPAIVs) from low pathogenicity viruses (LPAIVs) remain undefined. In July 2015, a disease investigation prompted by increased mortality on a commercial chicken farm, identified an incursion of H7N7 LPAIV that circulated within birds on the premises shortly before mutating to a HPAIV. HPAIV induced mortality in a subset of chickens, but the majority were protected, potentially due to prior exposure to LPAIV. Whilst genetic evidence for both H7N7 LPAIV and HPAIV were detected during the outbreak, only the HPAIV (A/chicken/England/26352/2015, H7N7-HP) was successfully isolated. To investigate the potential for protective responses being induced by prior LPAIV circulation, eight donor chickens were experimentally infected with A/mallard/Netherlands/19/2015 H7N7 LPAIV (H7N7-LP), a virus closely related to H7N7-HP, before eight contact chickens were introduced at 1 day post-infection (dpi). All eight donor chickens shed virus and seroconverted, whilst 50% of contact chickens shed virus and only 37.5% seroconverted, demonstrating H7N7-LP transmission. At 14 dpi, all 16 donor and contact chickens, alongside eight naïve positive control chickens, were challenged with H7N7-HP. From the control and contact groups, 100% and 87.5% of chickens shed H7N7-HP, with 62.5% and 50% mortality in these groups, respectively. None of the LP-donor chickens shed H7N7-HP or succumbed to infection. This demonstrated that infection of chickens with a LPAIV protects against infection with an antigenically-related HPAIV providing further insights into the clinical outcome during the outbreak. Analysis of these samples may help determine factors involved in LPAIV to HPAIV mutation.

## P274

# The effects of altering CpG composition in a virus genome are context-dependent

Colin Sharp[1], Sara Clohisey[1], Helen Wise[2], Douglas Stewart[3], Oliver Lin[1], Sam Wilson[3], Finn Grey[1], Peter Simmonds[4], Paul Digard[1], Eleanor Gaunt[1]
[1]The Roslin Institute, Edinburgh, United Kingdom. [2]Western General Hospital, Edinburgh, United Kingdom. [3]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [4]Oxford University, Oxford, United Kingdom

## Abstract

CpG dinucleotides are under-represented in the genomes of single-stranded (ss)RNA viruses. Adding CpGs to viral genomes causes virus attenuation, and this has led to suggestions that CpG enrichment can be used as a live attenuated vaccine development strategy. Recently, a cellular sensor of CpGs was identified. This protein, ZAP, is upregulated during interferon induction, which occurs during virus infection. We are using influenza A virus (IAV) as a model system to test the impact of CpG enrichment at various stages of virus replication, in order to understand whether CpG enrichment is a viable vaccine development strategy.

To test the effects of adding CpGs to a virus genome, we modified different regions of the 8-segmented IAV genome. Adding CpGs to either segments 1 (seg1-CpGH) or 5 (seg5-CpGH) caused impairment of viral replication. In ZAP knockout cells however, the deficiency in replication was reversed for seg1-CpGH but not seg5-CpGH viruses. The effects of CpG enrichment on transcription and translation for both constructs was minimal in cell-free and infection assays. Although CpG motifs in DNA are strongly associated with methylation, we found no evidence of altered methylation profile of CpG-modified viral RNAs. Serial passage of CpG-high viruses and thorough sequencing has revealed no reversion mutations occurring at CpG sites.

We support the suggestion that CpG enrichment can be used as a vaccine development strategy, but we must understand the impact of CpG enrichment on virus replication to be certain that there is no risk of reversion to a wildtype phenotype.

## P275

# Novel Rotavirus A genotypes discovered in bats and rats, and potential zoonosis from swine to humans in Vietnam

Jordan Ashworth, Lu Lu, Mark Woolhouse
Usher Institute, University of Edinburgh, Edinburgh, United Kingdom

## Abstract

Rotavirus is a leading cause of viral gastroenteritis worldwide, having the highest morbidity and mortality in children aged 5 and under. Rotavirus A (RVA) has a wide host range and is a known zoonotic pathogen. Here we assessed 2,100 faecal samples collected in Vietnam between 2012 and 2016 from enteric hospital patients, a variety of mammals (bats, swine, and rodents), and individuals with frequent occupational contact with animals, for the presence of RVA using metagenomic sequencing. 1/4 of hospital patients were positive for RVA, as well as several pigs, and for the first time in Vietnam, rats and bats. Phylogenetic analysis of segments 1 to 6 showed two novel host-specific genotype constellations in bats, as well as a novel VP4 genotype in rats. Several GXP[X] combinations were seen in humans, with the majority of sequences falling within human-specific lineages in genotypes G1P[8], G2P[4] and G8P[8]. Porcine genotypes G9P[6] and G4P[6] were also identified in humans. These sequences shared high identity to those seen in swine in Vietnam (>=96.6% nucleotide pairwise identity) and clustered phylogenetically with RVA sequences also isolated from humans and swine from elsewhere in Asia, including China and Japan. RVA is an important enteric pathogen in Vietnam, and while evidence of zoonotic transfer between humans and pigs was identified, the majority of genotypes clustered by isolation host. Further work would be needed to determine the zoonotic potential of novel genotypes discovered in bats and rodents in this study.

## P276

# Identification of Host Proteins that Interact with Non-Structural Proteins-1α and -1β of Porcine Reproductive and Respiratory Syndrome Virus-1

Sofia Riccio[1,2], Ben Jackson[1], Julian Hiscox[2], Simon Graham[1], Julian Seago[1]
[1]Pirbright Institute, Woking, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

## Abstract

Porcine reproductive and respiratory syndrome viruses (PRRSV-1 and -2) are the causative agents of one of the most economically important infectious diseases affecting the global pig industry. PRRSV produces 16 non-structural proteins (NSPs) and previous studies have shown that NSP1α and NSP1β modulate host cell responses; however, the underlying molecular mechanisms remain to be fully elucidated. Therefore, this project aims to identify and characterise novel PRRSV-1 NSP1-host protein interactions.

NSP1α and NSP1β from a representative Western European PRRSV-1 subtype 1 field strain were screened for interactions using a protein expression library generated from the primary target cell of PRRSV-1, porcine alveolar macrophages, and the yeast-2-hybrid (y-2-h) system.

The screens identified 62 and 127 putative binding partners for NSP1α and NSP1β, respectively. Three interactions from the NSP1α screen and 27 from the NSP1β screen were confirmed using y-2-h; these proteins are involved in either interferon signalling, the NF-κB pathway, ubiquitination, or nuclear transport.

Ongoing studies are characterising a selected number of novel interactions to increase our understanding of how PRRSV-1 NSP1α/β modulates the host cellular immune response, which could subsequently be exploited to rationally attenuate PRRSV-1 as a basis for improved vaccines.

## P277

# Failing to control Maedi-Visna

Scott Jones[1], Fiona Lovatt[1], Peers Davies[2], Stephen Dunham[1], Rachael Tarlinton[1]
[1]University of Nottingham, Nottingham, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

## Abstract

Maedi-Visna is a lentivirus of sheep that causes lung disease and chronic wasting. It has been designated an "Iceberg disease" by the UK sheep industry levy board with a very large burden of subclinical disease that is often not apparent until losses in an individual flock become catastrophic. Disease prevalence in the UK is thought to have doubled in the last 10 years, however farmer and veterinary awareness of the disease is poor. There is no vaccine and treatment is not cost effective, meaning that the only realistic control option is culling of affected animals.

Current testing protocols use MV gag protein ELISAs. A long lag time between infection and antibody production means that many animals are missed on flock screening and repeated rounds of testing over a period of years are necessary to remove all infected animals. Preliminary testing of flocks that have attempted eradication indicates that those that do not keep testing until all animals are negative fail to eliminate the disease and that prevalence rates can even increase substantially in these flocks. The viruses extreme variability confounded attempts to develop a qPCR capable of detecting all variants, indeed deep sequencing was required to establish which strains of virus are currently present in UK sheep as there has been substantial genetic drift since the last sequencing studies (performed more than 20 years ago). More promisingly virus was detectable in nasal swabs of experimental animals at least offering sampling methods that can be done by farmers themselves.

## P278

# The role of aberrant replication in mammalian infections with highly pathogenic avian influenza virus

Rebecca Penn, Cecilia Johansson, Wendy Barclay
Imperial College London, London, United Kingdom

## Abstract

Highly pathogenic avian influenza viruses (HPAIVs) pose a serious public health threat as they can transmit zoonotically with often fatal consequences. Zoonotic HPAIV infection has been associated with a dysregulated innate immune response known as a cytokine storm. We have previously demonstrated that a HPAIV virus with H5N1 internal genes (6:2 Tky05) triggered a cytokine storm in a mouse model. This virus was able to replicate to high levels in myeloid cells, triggering inappropriate levels of type I Interferons in a RIG-I and MAVS-dependent manner. Defective viral genomes (DVGs) which are generated by the viral polymerase in error during RNA replication are potent RIG-I ligands and it has been hypothesised that poorly adapted HPAIV polymerases may have a higher propensity to generate such aberrant RNA replication products in mammalian cells.

Our studies aim to elucidate the role that aberrant replication has in the mammalian cytokine storm. We generated stocks of the 6:2 Tky05 viruses that differed in their genome copy number to PFU ratio and show they contain DVGs. In lung epithelial cells the high DVG stock was extremely immunostimulatory at a very early time point post infection. DVGs were also amplified during virus replication in myeloid immune cells. In a mouse model infection with the high DVG 6:2 Tky05 virus resulted in a more severe phenotype with earlier weight loss and mortality than a virus with an identical genome constellation but lower level of DVGs. Our results suggest that immunostimulatory viral RNAs can contribute to influenza virus pathogenesis.

## P279

# Evaluation of different pseudotyping systems to aid lyssavirus vaccine development.

Bethany Auld[1], Mariliza Derveni[1], Thomas Simon[1], Pascale Schellenberger[1], Ashley Banyard[2], Edward Wright[1]
[1]University of Sussex, Brighton, United Kingdom. [2]Animal and Plant Health Agency (APHA), Weybridge, Surrey, United Kingdom

## Abstract

Without pre- or post-exposure prophylaxis, rabies virus causes an invariably fatal disease responsible for over 59,000 human deaths per year. Rabies virus is a lyssavirus, a genus comprising of 17 viral species all capable of causing rabies disease. The lyssavirus genus splits into three phylogroups based on genetic and antigenic properties. Studies have demonstrated that current rabies virus vaccines only confer protection against phylogroup I lyssaviruses indicating a novel vaccine is required to achieve coverage against all species.

A fundamental aspect of lyssavirus research is the use of pseudotype viruses (PV). Replication defective PV allow highly pathogenic viruses to be researched in category 2 laboratories, greatly enhancing the capability to investigate key research topics. PV exist as a viral core and lipid envelope bearing heterologous viral envelope proteins (VEP) on their surface. Different viral cores have distinct properties and so may provide better surrogates for VEP from different viral families. Here we have compared three commonly used pseudotyping systems bearing different viral cores - human immunodeficiency virus (HIV), murine leukaemia virus (MLV) and vesicular stomatitis virus (VSV) - in an attempt to identify a preferential surrogate for lyssavirus VEP. Two lyssavirus VEP from each phylogroup have been pseudotyped into each of the three systems. Purified PV have then been compared using infection assays and nanoparticle tracking to determine biological titres and quantities of potential non-functional particles. Results suggest the HIV core can provide a preferential surrogate, providing higher titres despite using less than one third of the glycoprotein DNA.

## P280

# Development of a lentivirus-mediated gene therapy targeting HIV-1 RNA to eliminate HIV-1-infected cells

Amanda Buckingham[1], Sophia Ho[1], Hoi-Ping Mok[1], Carin Ingemarsdotter[1], Andrew Lever[1,2]
[1]Department of Medicine, University of Cambridge, Cambridge, United Kingdom. [2]National University of Singapore, Yong Loo Lin School of Medicine, Singapore, Singapore

## Abstract

Only two patients have ever been cured of HIV-1. The latent HIV-1 reservoir is the major roadblock to this and necessitates lifelong therapy. In the 'shock and kill' approach to eliminate cells harbouring dormant virus the latency reversing agent (LRA) vorinostat increased HIV-1 RNA levels, but this failed to enable a reduction in reservoir size by immune- or virus-mediated cytotoxicity. To leverage LRAs' ability to heighten HIV-1 transcription, we are developing a 'kill' strategy that hijacks the HIV-1 splicing process with a therapeutic *trans*-splicing RNA, encoding an incomplete Herpes simplex virus thymidine kinase (HSV-tk) that gains functionality by *trans*-splicing onto HIV-1 tat pre-mRNA. HSV-tk activates the exogenous prodrug ganciclovir for selective killing of HIV-1-infected cells. Following proof-of-principle transfection studies, therapeutic constructs were engineered for lentivirus-mediated delivery to HIV-infected cells *in vitro*. We optimized lentiviral production for high infectious titer (>$10^6$ TU/mL) and low vector plasmid carryover (<1 copy/cell). In a tissue culture model of HIV-1 infection we confirmed lentiviral delivery of therapeutic (and control) vectors. Successful *trans*-splicing of the HSV-tk RNA onto HIV-1 RNA in cells co-transduced with HIV-1 and therapeutic vector at an MOI as low as 4 was confirmed by sequencing. In MTT assays the most potent therapeutic vector killed approximately 80% of HIV-1-expressing cells. Next-generation vectors are being evaluated for enhanced therapeutic potential and improved HIV-1-targeting RNA expression. The lead candidate culminating from this work will be assessed for selective killing of HIV-1-infected cells both productively infected and with virus reactivated from latency.

## P281

# Evaluation of bioinformatically designed, historical glycoproteins to aid lyssavirus vaccine development.

Bethany Auld[1], Simon Frost[2], David Wells[2], Ashley Banyard[3], Jonathon Heeney[2], Pascale Schellenberger[1], Edward Wright[1]
[1]University of Sussex, Brighton, United Kingdom. [2]Cambridge University Veterinary School, Cambridge, United Kingdom. [3]Animal and Plant Health Agency (APHA), Weybridge, Surrey, United Kingdom

## Abstract

Without pre- or post-exposure prophylaxis, rabies virus (RABV) causes an invariably fatal disease responsible for over 59,000 human deaths per year. RABV is a lyssavirus, a genus comprising of 17 viral species all capable of causing rabies disease. The lyssavirus genus splits into three phylogroups based on genetic and antigenic properties. Studies have demonstrated that current RABV vaccines only confer protection against phylogroup I lyssaviruses indicating a novel vaccine is required to achieve coverage against all species.

Bioinformatic analysis of evolutionary relationships between viruses can allow for genetic sequences of historic viral predecessors to be predicted. Historic sequences of immune eliciting proteins, such as glycoproteins (G), may be useful for designing vaccines of broader immunity than those employing single isolates. Whilst this has proven a useful tool for analysing closely related species, little has been done to determine if it's beneficial for a genus as broad as lyssaviruses. Here we have analysed all publically available lyssavirus G genes to generate a panel of 15 historic G. These were then expressed with the HIV pseudotyping system and the resulting pseudotyped viruses (PV) were used in infections assays to determine protein functionality. Two thirds of the G were functional and various attempts to improve this number were unsuccessful. Functional historic PV and PV bearing G from current vaccine isolates were then subject to comparative neutralisation assays with selective sera specifically targeting lyssavirus species/phylogroups. Results from these assays revealed specific neutralisation sensitivities that could aid the development of a pan-lyssavirus vaccine.

## P282

# Respiratory pathogens co-infection in patients with COVID-19 pneumonia in Kazakhstan.

Alyona Lavrinenko, Svetlana Kolesnichenko, Anar Turmukhambetova, Irina Kadyrova
Karaganda Medical University, Karaganda, Kazakhstan

## Abstract

Background. Clinical evidence of the presence of bacterial co-infection in patients with SARS-CoV-2 pneumonia is important for an adequate treatment with antibacterial drugs. Objectives: to determine the secondary bacterial flora in patients with COVID pneumonia in patients in the Republic of Kazakhstan.

Methods. Prospective, microbiological, multicenter study, which was conducted at the Medical University of Karaganda in the Shared Resource Laboratory. Sputum samples were collected from three cities of Kazakhstan with the worst SARS-CoV-2 epidemiological situation. Microbiological examination was carried out using classical methods. All investigated isolates were identified to species by MALDI-TOF mass spectrometry. Susceptibility to antibiotics was performed by the disk diffusion method in accordance with the CLSI 2019 recommendations.

Results. 133 patients were included with a mean age of 60.9±12.7 years old, 53/133 (39.8%) had confirmed SARS-CoV-2 PCR test. Microbiological examination showed the growth of normal microflora in 31.45%. Difference in secondary bacterial co-infection etiology in COVID-19 positive and negative patients was not found. The leading place in general etiological structure belonged to *K.pneumoniae* - 22.64%, *E.coli* - 11.95% and *A.baumannii* - 11.32%. A significant relationship ($p = 0.003$) between such parameters as the use of antibacterial drugs and the isolated microflora was found.

Conclusions. Isolated microorganisms are etiologically significant and are pathogens of the ESKAPE group. It is important to limit the risk of infection and spread of resistant microorganisms by closely monitoring nosocomial infections and drawing attention to secondary infections caused by resistant bacteria that can increase the mortality rate of patients with COVID-19.

## P283

# SARS-coV-2 infection in the neonate: A systematic review

Paul Flinders, Jasim Shihab, Hannah Brophy
NICU, Burnley General Teaching Hospital, East Lancashire Hospitals NHS Trust, Casterton Avenue, Burnley, United Kingdom

## Abstract

### Background

The worst effects of the Sars-CoV2 pandemic have fallen on the very eldest in society, with children being relatively spared. However comparatively less is known about the clinical features and outcomes with respect to neonatal patients with Sars-CoV2. With the related Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) known to cause adverse neonatal outcomes, there is concern that Sars-CoV2 will have similar consequences.

### Methods

In November 2020, a systematic search of PubMed, MEDLINE and Embase was conducted to identify original studies investigating the presentation of Covid-19 in neonates. The search terms (covid-19 OR coronavirus OR SARS-CoV-2) AND (neonate OR newborn OR neonatal) were used. From 1363 initial articles, 71 relevant studies were identified.

### Results

211 Sars-CoV2 positive neonates were identified. The most common symptoms were fever (46.8%, n=94/201), tachypnoea (31.8%, n=64/201), hypoxia (31.8%, n=64/201), lethargy (19.9%, n=40/201) and rhinorrhoea (17.4%, n=35/201). 13.9% (n=28/201) of neonates were asymptomatic. NICU admission was required in 55.8% (n=101/181) with respiratory support being delivered in 60.2% (n=112/186) of cases. 15.1% (n=28/186) needed the highest level of support in the form of intubation and ventilation. 16 neonates (7.6%) met the criteria for vertical transmission with positive PCR tests within 12 hours of birth. Four neonates died with covid (1.9%), although all deaths were attributable to other causes.

### Conclusion

Fever and respiratory symptoms were the most common presentation of Sars-CoV2 in neonates. No neonatal deaths were solely attributable to Sars-CoV2.

## P284

# Potentials of Breast Milk Antibodies as a Therapeutic for COVID-19

VICTORY NNAEMEKA
UNIVERSITY OF NIGERIA, NSUKKA, NSUKKA, Nigeria

## Abstract

Since the explosion of pneumonia-like symptoms in the province of Hubei, Wuhan in December 2019 over 200 countries have gotten a share of this disease caused by the novel coronavirus disease 2019 (COVID-19). Till date, there exists no modest therapeutic agent to combat this virus. Antibodies in the breast milk of convalescent lactating mothers could be the silver bullet that would deflate the ballooning morbidity and mortality rate caused by the COVID-19 pandemic. Coupled with the fact that breast milk contains a very high amount of Immunoglobulin A which has a desirable higher in vivo stability than other antibodies. This novel approach of purifying antibodies from the breast milk of convalescent lactating mothers is a non-invasive technique relative to the conventional method of using convalescent plasma which had proven effective for generating passive immunity against the seasonal flu. Purified Immunoglobulins would be obtained by adding an equal volume of ammonium sulphate solution to the breast milk sample to induce a lyotropic effect and thereafter passing the partially purified solution through a column chromatographic system impregnated with a Jaclin –lectin, Protein A, L and G. Neutralizing antibodies would by assayed using the Enzyme-Linked Immunosorbent Assay technique. Various statistical tools will be used to analyze a double-blinded randomized clinical trials to ascertain the efficacy of the purified antibodies as it is expected to have clear cut effectiveness over the control and potentially become the invaluable in containing the pandemic.

## P285

# Bat Interferon Regulatory Factor 7 Displays Potential Auto-Translocating Ability

Emily Clayton, Muhammad Munir
Lancaster University, Lancaster, United Kingdom

## Abstract

Bats are major natural reservoirs of zoonotic viruses and possess the ability to host several viruses without exhibiting signs of disease, owing to novel immune mechanisms. My research aims to identify key elements of the bat innate immune system that allow limitation of viral immunopathology, focusing primarily on components of the bat interferon system.

The IRF7 is a central regulator crucial to the interferon response in humans and is functionally conserved in bats. To investigate the role of IRF7 in the innate immune cascade of *P.alecto* at the cellular level, HEK293T cells were transfected with *P. alecto* IRF7 and selected cells were stimulated with GFP-expressing VSV (Vesicular stomatitis Indiana virus). Immunofluorescence staining was undertaken using a primary antibody raised against the FLAG tag-*P.alecto* IRF7 and visualized using confocal microscopy.

Part of the interferon cascade involves the phosphorylation and dimerization of IRF7 in the cell cytoplasm and its successive translocation to the nucleus where it acts as a transcription factor for the production of interferons. As anticipated, when stimulated with VSV, *P.alecto* IRF7 had translocated from the cytoplasm and was visible in the cell nucleus only. However, it was also observed that *P.alecto* IRF7 had translocated to the nucleus in unstimulated cells.

Results propose the potential auto-translocating ability of *P.alecto* IRF7, suggesting the capacity of bats to activate transcription of interferons in absence of viral stimuli. Further structural and functional investigations are envisaged. These observations may confer an advantageous role to bats in accommodating viruses without exhibiting ostensible signs of disease.

## P286

# Longevity and neutralisation activity of secretory IgA following SARS-CoV-2 infection

Samuel Ellis[1], Alice Burleigh[1], Rosie Way[1], Japhette Kembou-Ringert[1], Tereza Masonou[1], Kimberly Gilmour[2], Louis Grandjean[1,2], Claire Smith[1]
[1]University College London, London, United Kingdom. [2]Great Ormond Street Hospital, London, United Kingdom

## Abstract

The mucosal barrier is a primary defence against inhaled pathogens, comprising secretory antibodies which have the potential to block viral entry and neutralise infection. There is an ongoing need for greater understanding of the mucosal immunity to SARS-CoV-2 infection. In this study, we investigated mucosal IgA through non-invasive saliva sampling of healthcare workers.

A total of 551 saliva samples were collected from staff at Great Ormond Street Children's Hospital who previously tested positive for COVID-19. Participant metadata included age, gender, ethnicity and symptoms. IgA titres were measured by ELISA against viral antigens spike protein, nucleocapsid protein, and spike receptor-binding domain. SARS-CoV-2 neutralisation was measured using a VERO E6 cell culture infection assay.

We found that approximately 30% of saliva samples contained detectable IgA specific for at least one of the SARS-CoV-2 antigens. IgA levels in saliva decreased with the time post-infection, and were largely undetectable after six months. IgA titres specific to SARS-CoV-2 were lowest in participants over 60 years old. Specific saliva samples were identified which effectively neutralised SARS-CoV-2 virus infection of epithelial cells.

Our results suggest secretory IgA specific to SARS-CoV-2 can be detected in saliva following infection, an accessible sample type for testing, although titres decreased over time. Some saliva samples were able to neutralise SARS-CoV-2 infectivity against cultured epithelial cells. This data could be used to assess the risk of re-infection with SARS-CoV-2, as well as accelerate efforts to develop effective mucosal vaccination with longer lasting protection.

## P287

# Differential infectivity of SARS-CoV-2 in primary airway epithelium derived from different donors

Lindsay Broadbent, Connor Bamford, David Courtney, Ahlam Ali, Olivier Touzelet, Ken Mills, Ultan Power
Queens University Belfast, Belfast, United Kingdom

## Abstract

SARS-CoV-2, the virus that causes COVID-19, was identified in late 2019 and went on to cause over 1.5 million deaths in a year. The spectrum of COVID-19 disease ranges from asymptomatic or sub-clinical to severe long term damage to multiple organ systems or death. The reasons for varying responses to SARS-CoV-2 infection remain to be elucidated.

Using our well-differentiated primary airway epithelial cell (WD-PAEC) model, which authentically replicates the morphology and physiology of the human airway epithelium, we investigated the host response to a recent isolate of SARS-CoV-2. Interestingly, we discovered that but not respiratory syncytial virus (RSV) infection, varied in WD-PAECs from different donors. Indeed, 3 donors were highly susceptible, while a 4$^{th}$ was not. High baseline levels of secreted IFNλ coincided with resistance to SARS-CoV-2 infection. Interestingly, although endogenously secreted IFNλ concentrations were high in resistant donor, chemokine and cytokine secretions following infection were not significantly different from the susceptible donors. IFNλ treatment of WD-PAECs from a donor susceptible to infection resulted in greatly reduced SARS-CoV-2 titres.

These results indicate that susceptibility to SARS-CoV-2 infection varies between donors. These data may explain, in part, differential susceptibility to COVID-19 disease following exposure to SARS-CoV-2.

## P289

# SARS-CoV-2 triggers an MDA-5-dependent interferon response which is unable to control replication in lung epithelial cells

Antoine Rebendenne[1], Ana-Luiza Chaves Valadão[1], Marine Tauziet[1], Ghizlane Maarifi[1], Boris Bonaventure[1], Rémi Planès[2], Joe McKellar[1], Sébastien Nisole[1], Mary Arnaud-Arnould[1], Olivier Moncorgé[1], Caroline Goujon[1]
[1]IRIM, Montpellier, France. [2]IPBS, Toulouse, France

## Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiologic agent of coronavirus disease 19 (COVID-19), which ranges from mild respiratory symptoms to acute respiratory distress syndrome, and death in the most severe cases. Immune dysregulation with altered innate cytokine responses is thought to contribute to disease severity.

Here, we characterized in depth host cell responses to SARS-CoV-2 infection, using primary human airway epithelia (HAE). Our results demonstrate that primary HAE elicit a robust induction of type I and III interferons (IFNs) genes and secretion of these cytokines in the basal media. However, this response arrived late upon viral exposure. The naturally permissive Calu-3 lung cell line recapitulated host cell response to SARS-CoV-2 infection, and, particularly, IFN induction, which validated the use of this model cell line to study viral replication and induction of innate immunity.

Interestingly, HAE and Calu-3 cells could inhibit SARS-CoV-2 replication and *de novo* production of infectious virions upon type I IFN pre-exposure. Using Calu-3 cells, we further showed that melanoma differentiation-associated protein 5 (MDA-5) was the main innate immune sensor of SARS-CoV-2 in these epithelial cells. Finally, we demonstrated that, despite high levels of type I and III IFNs produced in response to SARS-CoV-2 infection, the IFN response was unable to control viral replication in human lung cells, contrary to what was previously reported in intestinal epithelial cells.

Altogether, these results highlight the complex and ambiguous interplay between viral replication and the timing of IFN responses.

## P290

# Comparison of lentiviral and vesicular stomatitis virus core SARS-CoV-2 pseudotypes and generation of a stable cell line for use in antibody neutralisation assays

Martin Mayora Neto[1], Diego Cantoni[1], Cecilia di Genova[1], Simon Scott[1], Mariliza Derveni[2], Edward Wright[2], Nigel Temperton[1]
[1]Viral Pseudotype Unit, Kent, United Kingdom. [2]Viral Pseudotype Unit, Falmer, United Kingdom

## Abstract

Betacoronavirus SARS-CoV-2, the causative agent of COVID19, is a single stranded positive sense RNA virus. Since its emergence there has been great efforts to identify correlates of protection**,** which is crucial for vaccine evaluation studies. However, handling SARS-CoV-2 requires BSL-3 containment facilities slowing research efforts. Pseudotype viruses (PV) are a safe alternative to authentic virus that can be handled at low containment. PVs are chimeric viruses containing the core of a virus where its genome has been completely or partially replaced by a reporter gene, displaying a correctly folded SARS-CoV-2 spike on its surface. We developed lentiviral and vesicular stomatitis virus (VSV) core PVs alongside a stable A549 cell line expressing receptor ACE2 and protease TMPRSS2 responsible for S protein priming, for use in neutralization assays. Lentiviral PVs were generated by transfection with plasmids encoding the spike, HIV-1 gag-pol and a luciferase reporter. For VSV PVs, producer cells pre-transfected with the spike were infected with recombinant VSV expressing luciferase, before harvesting. The stable A549 cell line was generated by sequential infection of VSV-G PVs bearing lentiviral vectors encoding ACE2 and TMPRSS2 genes followed by antibiotic selection, before being tested in neutralization assays. We compared lentiviral and VSV PV platforms using monoclonal antibodies and convalescent sera with our stable A549 cells or HEK293T cells pre-transfected with plasmids encoding ACE2 and TMPRSS2. Antibody titres showed equivalence however VSV had the advantage of a shorter incubation therefore enabling a higher throughput. PVs offer a robust platform for future seroepidemiology and vaccine evaluation studies.

## P291

# Contribution of SARS-CoV-2 genomic RNA sequence structures to encapsidation and packaging

Kayden Xie[1], Amanda Buckingham[1], Carin Ingemarsdotter1[1], Fanny Salasc1[1], Aaron D'Souza[1], Andrew Lever[1,2], Julia Kenyon#[1,2,3], Harriet Groom#[1,4]
[1]University of Cambridge, Cambridge, United Kingdom. [2]National University of Singapore, Singapore, Singapore. [3]Homerton College, Cambridge, United Kingdom. [4]Sidney Sussex College, Cambridge, United Kingdom

## Abstract

During SARS-CoV-2 assembly, the viral genomic RNA is encapsidated by viral nucleocapsid proteins and selectively packaged over viral subgenomic and host RNA species. In coronaviruses, this packaging selectivity is thought to be conferred by specific viral protein-mediated recognition of RNA packaging signals, which are conserved structures and sequences unique to the viral genome. However, the identity of the SARS-CoV-2 packaging signal remains elusive. This project aims to identify the SARS-CoV-2 genomic RNA secondary structures and sequences important for recognition by the nucleocapsid protein. To this end, using gel-shift assays, we show that the nucleocapsid protein can bind the 5'-end of the viral genome with multi-step kinetics. Using UV-crosslinking-coupled SHAPE (XL-SHAPE), we are mapping the 5'-end genomic sites to which the nucleocapsid protein binds and following the RNA structural rearrangements which occur upon nucleocapsid protein binding. From our results, we propose a model which describes how SARS-CoV-2 nucleocapsid protein may capture the viral genome and facilitate encapsidation and subsequent packaging. Our work paves the way for the design of antisense oligonucleotides and/or assays for the screening of RNA-binding compounds which disrupt SARS-CoV-2 encapsidation and packaging.

[1]These authors contributed equally

# corresponding authors

## P292

# SARS-COV-2 VLPs expressed by the Baculovirus System as a reagent for vaccine and diagnostic use.

Edward Sullivan[1], Weining Wu[1], Po-Yu Sung[1], Ian Jones[2], Polly Roy[1]
[1]London School of Hygiene and Tropical Medicine, London, United Kingdom. [2]University of Reading, Reading, United Kingdom

### Abstract

Virus-like-particles (VLPs) are macromolecular assemblies that resemble virus particles. They are formed by heterologous expression of the minimum number of virus structural proteins required for virus assembly but can also incorporate additional viral proteins if they are compatible with the assembly process. VLPs are non-infectious as no genome is present and can serve as models for the assembly process or as surrogates for authentic viruses for antibody binding or antibody generation. Importantly their maintenance of a relevant tertiary structure offers the potential to bind and induce conformational antibodies that are not possible when individual protein subunits are used. Further, their regular array-like presentation can cross link B-cell receptors to generate more potent antibody responses.

Previously we have shown that SARS VLPs can be generated by co-expression of the M and E proteins and that the Spike (S) protein is incorporated when co-expressed. Here we show that baculovirus expression of S, M and E of the SARS-CoV-2 leads to efficient VLP formation. VLPs were secreted from baculovirus infected cells and purified by velocity or density gradient centrifugation. VLPs were highly enriched in SARS-CoV-2 structural proteins and appeared as classical morphologies when examined by electron microscopy. VLPs bound a human monoclonal antibody specific for the receptor binding site of S and demonstrated superior sensitivity on a weight for weight basis with S alone when used as a diagnostic antigen in standard ELISA format.

We conclude that SARS-CoV-2 VLPs are potentially significant reagents for diagnostic and candidate vaccine use for SARS-CoV-2 and COVID-19.

## P294

# COVID-19: Modelling SARS-CoV2 Viral Variant Disease Pathology.

Debbie Ferguson[1], Sarah Kempster[1], Jo Hall[1], Claire Ham[1], Adrian Jenkins[1], Yemisi Adedeji[1], Vicky Rannow[1], Elaine Giles[1], Rose Leahy[1], Sara Goulding[1], Arturo Fernandez[1], Yann Le Duff[1], Robert Francis[1], Kevin Bewley[2], Karen Osman[2], Naomi Coombes[2], Simon Funnell[2], Neil Berry[1], Neil Almond[1]
[1]NIBSC, Potters Bar, United Kingdom. [2]PHE, Salisbury, United Kingdom

## Abstract

It is apparent that COVID-19 disease is not limited to the respiratory system and systemic pathologies of variable severity. Animal models enable the role of direct viral and indirect pathologies to be discriminated to inform improved treatments and optimising immune based therapies.

Four animal species (Syrian Hamsters, Cynomolgus Macaques, Red Bellied Tamarins and Common Marmosets) were infected intranasally with SARS-CoV2 isolate Victoria-01. Syrian hamsters were infected intranasally with isolate B.1.1.7. Following termination of pairs of each species 2, 10 or 28 dpi FFPE tissue sections were analysed for the presence of viral RNA (RNAscope), viral proteins (immunohistochemistry) and pathology (H+E).

Victoria-01 was detectable within the upper and lower respiratory tract of all species except common marmosets. Infected cells at 2dpi were predominantly epithelial cells of the bronchovasculature with virus subsequently detected in regions of inflammation.

Highest levels of an inflammatory pathology including blood clotting were seen 2dpi with a subsequent development of oedema at 10 and 28dpi.

Virally infected cells and inflammatory infiltrates were extensively present within lungs of Syrian hamsters which at 28dpi developed into large syncytia staining strongly for spike protein when virus was not detectable in the upper respiratory tract.

B.1.1.7 was detectable within the broncovasculature and inflammatory clusters of hamster lungs 2dpi. Further analyses are ongoing.

This detailed characterisation of pathology following infection will enable the beneficial impact and limitations of immune based medicines and other therapeutics to be fully understood and identify where improvements may be made.

## P295

# SARS-CoV-2 Spike has broad tropism for mammalian ACE2 proteins yet exhibits a distinct pattern of receptor usage when compared to other β-coronavirus Spike proteins.

Carina Conceicao*[1], Nazia Thakur*[1], Stacey Human[1], James T Kelly[1], Leanne Logan[1], Dagmara Bialy[1], Sushant Bhat[1], Phoebe Stevenson-Leggett[1], Adrian K Zagrajek[1], Philippa Hollinghurst[1,2], Michal Varga[1], Christina Tsirigoti[1], Matthew Tully[1], Chris Chiu[1], Katy Moffat[1], Adrian Paul Silesian[1], John A Hammond[1], Helena J Maier[1], Erica Bickerton[1], Holly Shelton[1], Isabelle Dietrich[1], Stephen C Graham[3], Dalan Bailey[1]
[1]The Pirbright Institute, Woking, United Kingdom. [2]Department of Microbial Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom. [3]Department of Pathology, University of Cambridge, Cambridge, United Kingdom

## Abstract

The Coronavirus Disease 2019 (COVID-19) pandemic, caused by SARS Coronavirus 2 (SARS-CoV-2), continues to cause significant mortality in human populations worldwide. SARS-CoV-2 has high sequence similarity to SARS-CoV and other related coronaviruses circulating in bats. It is still unclear whether transmission occurred directly from bats to humans, or through an intermediate host, bringing into question the broader host range of SARS-CoV-2. Using a combination of low biocontainment entry assays as well as live virus, we explored the receptor usage of SARS-CoV-2 using angiotensin-converting enzyme 2 (ACE2) receptors from 22 different species. We demonstrated that in addition to human ACE2, the Spike of SARS-CoV-2 has broad tropism for other mammalian ACE2s, including dog, cat and cattle. However, comparison of SARS-CoV-2 receptor usage to the related SARS-CoV and bat coronavirus, RaTG13, identified distinct patterns of receptor usage, with the two human viruses being more closely aligned. Finally, using bioinformatics, structure analysis and targeted mutagenesis, we identified key residues at the Spike-ACE2 interface which may have played a pivotal role in the emergence of SARS-CoV-2 in humans, some of which are also mutated in newly circulating variants of the virus. To summarise, the broad tropism of SARS-CoV-2 at the point of viral entry identifies the potential risk of infection of a wide range of companion animals, livestock and wildlife.

## P296

# Seroprevalence of SARS-CoV-2, HCoV-NL63, HCoV-229E and HCoV-HKU1 neutralising antibodies in a cohort of hospital staff and patients from the United Kingdom

Diego Cantoni[1], Martin Mayora-Neto[1], Cecilia Di Genova[1], Alex Sampson[2], Andrew Chan[2], George Carnell[2], David Wells[2], Helen Baxendale[3], Javier Castillo-Olivares[2], Jonathan Heeney[2], Nigel Temperton[1]
[1]Viral Pseudotype Unit, University of Kent, Medway, United Kingdom. [2]Laboratory of Viral Zoonotics, Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom. [3]Royal Papworth Hospital NHS Foundation Trust, Cambridge, United Kingdom

## Abstract

The SARS-CoV-2 virus, causative agent of the COVID-19 disease, has rapidly spread around the world, causing more than 100 million cases and over 2 million deaths. Infection cases have shown ranges of severity in disease, from asymptomatic to severe, leading to death. As a result, much focus has been placed on assessing the immune response to the virus, and in particular, the antibody titres between different categories such as severity of disease and age. The seasonal coronaviruses HCoV-229E, HCoV-NL63 and HCoV-HKU1 are close relatives of SARS-CoV- 2, however, not much is known as to whether previous exposure to these viruses may produce cross reactive neutralising antibodies to SARS-CoV-2. In addition, NL63 requires ACE2 receptor for binding and entry, like SARS-CoV-2. We pseudotyped the three seasonal coronaviruses; HCoV-NL63, HCoV-HKU1, HCoV-229E and SARS-CoV-2, using a lentiviral based system approach with HEK293T cells, to assess the levels of neutralising antibodies present in a cohort of patients and hospital staff in the UK with and without a history of SARS-CoV-2 infection. In total, 101 patient and staff sera were screened against all four pseudotyped viruses. We detected neutralising antibodies of the three seasonal coronaviruses in both patient and staff samples, including sera from asymptomatic individuals. However, our results suggest that previous exposure to the seasonal coronaviruses did not confer any protection towards SARS-CoV-2, including ACE2 interacting HCoV-NL63.

## P297

# Engineering a Vaccine Platform using Rotavirus A to Express SARS-CoV-2 Peptides

Victoria Gonzalez[1], Luca Venditti[2], Olga Lee[1], Colin Sharp[1], Sarah Caddy[3], Paul Digard[1], Alexander Borodavka[2], Eleanor Gaunt[1]
[1]The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom. [2]Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom. [3]Department of Medicine, University of Cambridge, Cambridge, United Kingdom

## Abstract

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a pressing need for efficacious vaccines. Upon infection, SARS-CoV-2 enters both the respiratory and gastrointestinal tract. Rotavirus A (RV-A) infects the gastrointestinal tract, with cross-immunization of respiratory and gastrointestinal sites likely. The goal of this study is to engineer vaccine candidates using RV-A as a delivery platform for the expression of SARS-CoV-2 spike peptides.

Using plasmid mutagenesis, spike epitopes from the receptor-binding domain which have previously been demonstrated to invoke strong antibody responses were introduced into the variable region of simian rotavirus SA11 VP4, a highly immunogenic surface protein that leads to the production of antibodies upon infection. Five mutant SA11 viruses expressing spike peptides were successfully rescued. The expression of the SARS-CoV-2 spike epitopes had little effect on virus replication, as a minimal drop in titre was observed compared to wild type SA11. Further characterization of replication kinetics using time-course experiments, protein production using western blots and confocal imaging, and RNA assays using gel electrophoresis and qPCR are ongoing. Preliminary findings look promising and we will next investigate the immune response invoked by the SARS-CoV-2 spike epitopes. This study may lead to the successful engineering of RV-A expressing SARS-CoV-2 spike peptides which can be taken forward for further development as vaccine candidates. As RV vaccines can be multiplexed to offer protection against multiple strains, this principle could be applied here to target multiple spike peptides or SARS-CoV-2 variants.

## P298

# Deciphering the interaction between ORF6 from SARS-CoV-2 and endogenous nuclear transporters using NMR spectroscopy.

Lydia S. Newton[1], Morten L. Govasli[2], Greg J. Towers[2], D. Flemming Hansen[1]
[1]Institute of Structural and Molecular Biology, Division of Biosciences, University College London, London, United Kingdom. [2]Division of Infection and Immunity, University College London, London, United Kingdom

## Abstract

The SARS-CoV-2 genome encodes accessory proteins that have evolved to conceal viral replication from host antiviral responses. One such protein, ORF6, has previously been shown to antagonise type I interferon (IFN) responses and to inhibit downstream signalling. ORF6 has been proposed to exert its antagonising effect by binding and inhibiting endogenous nuclear transporters of the importin-α family such as KPNA2. Furthermore, the ability of ORF6 to suppress host innate immunity has been functionally mapped to its C-terminal region.

Here we used nuclear magnetic resonance (NMR) spectroscopy to characterise the ORF6 C-terminal and its interactions with KPNA2. A $^{1}H$, $^{13}C$ chemical shift assignment of the C-terminal domain of ORF6 (residues 41-61) was obtained from two-dimensional $^{1}H$-$^{1}H$ TOCSY, $^{1}H$-$^{1}H$ NOESY and $^{1}H$-$^{13}C$ HSQC NMR spectra. Secondary chemical shifts consistent with random coil and the absence of long-range NOEs established that the C-terminal domain lacks secondary structure, stable long-range interactions and tertiary structure. Despite the lack of structure, residue-specific chemical shift perturbations were observed in ORF6 spectra upon addition of KPNA2, thereby confirming a direct interaction between the two proteins. These chemical shift perturbations were considerable for amino acids near the C-terminus of ORF6, including E59, I60 and D61.

This work provides novel insight into the interaction between SARS-CoV-2 ORF6 and KPNA2 and lays the foundations for further characterisations of ORF6 and its interaction with endogenous proteins. An understanding of the molecular mechanisms mediating the pathogenesis of SARS-CoV-2 will undoubtedly benefit the development of future treatments against SARS-CoV-2 and similar coronaviruses.

## P299

# Structural and Evolutionary Insights into the Binding of Host Receptors by the Rabies Virus Glycoprotein

Manar E. Khalifa, Leonie Unterholzner, Muhammad Munir
Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster, United Kingdom

## Abstract

Rabies represents a typical model for spill-over of zoonotic viral diseases among different hosts. Understanding the mechanisms by which RV switch among hosts requires analysis of viral evolution and host interactions. To understand the global colonization of RV, we constructed phylogenetic trees using 283 sequences of glycoproteins (G) of RV with three different algorithms. Our analysis revealed a host-specific speciation and distribution of RV across the globe. In conjunction, we investigated the structural analysis and sequence alignment of host receptors among different RV-susceptible species. One of the major findings in our study was the absence of integrin plexin domain in the ITGB1 receptor of the black fruit bat. Interestingly, the nAChR interaction site with RV G protein was conserved among different species. For studying the interaction dynamics between RV G protein and the RV receptors, we constructed 3D structures for RV receptors and G proteins using homology modelling. Different servers were implied for protein-protein docking between RV G protein and receptors highlighting the variability of interacting residues between different species of RV receptors. The absence of *invitro* model system for RV has limited the analysis of RV entry mechanisms. Therefore, we report establishment of *invitro* system based on expressing RV receptors, together with establishment of reverse genetics system to rescue recombinant VSV with RV-G gene. This system provides a powerful tool for analysis of RV-G with different receptors. Overall, this study provides the foundation of future host-virus interaction studies to underpin the importance of receptor-mediated spill-over events in RV infections.

## P300

# Exploring foot-and-mouth disease virus antibody interactions using biolayer interferometry

Andrew Shaw[1], Alison Burman[1], Amin Asfor[1], Anna Ludi[1], Emiliana Brocchi[2], Santina Grazioli[2], Donald King[1]
[1]The Pirbright Institute, Pirbright, United Kingdom. [2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy

## Abstract

Foot-and-mouth disease virus (FMDV) vaccines protect animals from infection by inducing antibodies. The level of neutralising antibody induced in response to vaccination (or infection), as measured by a virus neutralisation test, is an important parameter with regards to the level of protection afforded against subsequent challenge. However, in addition to overall titre, antibody avidity also represents a crucial metric when assessing the protective efficacy of antibodies. In this project we investigated the use of biolayer interferometry (BLI) to measure the avidity of FMDV antibodies to FMDV antigens. Antibodies targeting site I of the FMDV particle were detected using a commercially synthesised biotinylated peptide. In contrast, the entire antigenic landscape of the FMDV particle was represented by biotinylated FMDV capsids. The antigens were loaded onto Octet streptavidin biosensors at an optimal concentration prior to dipping into antibodies. The sera from different animals varied in avidity, reflecting the quantitative differences in avidity that exist between individual animals in response to FMDV vaccines. Interestingly, the Kdis values obtained for site I vs the entire capsid were different, supporting the importance of other sites beyond site I. Similarly, monoclonal antibodies targeting distinct, known antigenic sites on the capsid surface also resulted in different avidities. The BLI methodology reported here offers a useful approach by which to investigate the strength of antibody interactions at specific sites. In conjunction with recombinant technology, BLI will help aid in investigations into the relative importance of the different antigenic sites with regards to inducing a protective response.

## P301

# The N-terminal domain of MX1 proteins is essential for their antiviral activity

Joe McKellar[1], Mary Arnould[1], Marine Tauziet[1], Laurent Chaloin[1], Charlotte André[1], Wendy S. Barclay[2], Georg Kochs[3], Caroline Goujon[1], Olivier Moncorgé[1]
[1]Institut de Recherche en Infectiologie de Montpellier (IRIM), CNRS, Montpellier, France. [2]Imperial College London, Section of Virology, Department of Medicine, London, United Kingdom. [3]Institut fuer Virologie, Freiburg, Germany

## Abstract

Cells infected by a virus produce interferon (IFN) which in turn induces an antiviral state through the expression of several hundred IFN-stimulated genes (ISGs). The Myxovirus resistance proteins 1 and 2, MX1 and MX2 are dynamin-like large GTPases and these ISGs contribute to a significant proportion of the antiviral IFN-induced effect. MX1 proteins are highly conserved amongst mammals and human MX1 has been shown to elicit antiviral activity against a wide variety of RNA and DNA viruses, most notably influenza A virus (IAV), acting at various steps of their viral lifecycles. The process through which MX1 proteins are able to inhibit these viruses is still not known, despite over fifty years of efforts. Nevertheless, it has been shown that MX1 needs a functional GTPase domain, the ability to oligomerize through its stalk domain, a complete Bundle Signaling Element and intact L2 and L4 loops for its antiviral activity. MX1 proteins also possess a non-structured N-terminal domain that varies in length between species, of which the role in their antiviral activity has not been elucidated. Here, through the use of mutagenesis, microscopy and biochemical studies, we show that the N-terminal domain of MX1 proteins, more specifically a single amino acid, is essential for their antiviral activity against various RNA viruses, such as IAV. The discovery of this essential and conserved residue may provide us with new insights to help us understand the mechanism of action of these impressive antiviral proteins.

## P302

# Investigating the sub-nuclear localisation of Influenza A Virus replication

Olivia Swann, Thomas Peacock, Wendy Barclay
Imperial College, London, United Kingdom

## Abstract

Influenza A virus (IAV) replicates in the nucleus of infected cells. This involves both transcription from the negative sense RNA viral genome (vRNA) to produce capped and polyadenylated viral messenger RNAs (mRNA), and replication of the genome, which occurs in a two-step process via a full-length complementary genome intermediate (cRNA). Both transcription and replication are directed by the same RNA dependent RNA polymerase (FluPol), despite differing mechanistically. Interactions of the FluPol with different host factors are associated specifically with transcription (vRNA to mRNA) or replication (either vRNA to cRNA or cRNA to vRNA). For example, the host protein ANP32 has been linked to cRNA to vRNA replication. However how these interactions are regulated to direct timely and appropriate transcription and replication is less well understood.

To investigate the regulation of FluPol, we have developed infection assays utilising RNAscope probes that are able to specifically detect IAV cRNAs and vRNAs. Utilising this approach, we have gathered evidence suggesting ANP32 has a role in v to cRNA synthesis, in addition to c to vRNA. This conclusion is further supported by qRT-PCR analysis. Using cRNA and vRNA as markers of FluPol replication, we are now investigating whether viral replication can be localised to a specific subnuclear niche. We are also investigating whether aberrant localisation of avian-origin FluPol may contribute to host restriction of these viruses in mammalian cells.

## P303

# Investigating the microbiological risks associated with urban flooding in the UK

Sophie Scutt, James Shucksmith, Isabel Douterelo
University of Sheffield, Sheffield, United Kingdom

## Abstract

Over the last 30 years, the frequency and occurrence of intense rainfall, and thus extreme hydrological events –flooding- has steadily increased. Drainage infrastructure in the UK was not designed for a changing climate, and many sewer systems in densely populated urban areas, are unable to cope. Sewage overflow and surface run off in urban areas can act as vectors for the dissemination of pathogens, known to cause disease among human populations.Most of the previous studies in this field have focused on using faecal indicators such as *E.coli* when assessing the public health risk of floodwater [1]. However, traditional indicators do not accurately reflect the true risk that urban flooding poses [2]. Little is understood in regards to the survivability and behaviour of pathogens in different urban settings, which are fundamental to determine potential risks to public health.

Previous investigations in UK waterlogged soils have shown a clear response of microbial communities to water table variation, temperature, and nutrient availability in soil profiles [3]. This research aims to investigate, using advanced molecular methods, the dynamics of pathogens (i.e. movement through soil and survival rates), and microbial interactions at the soil/water interface- collecting information from field work studies and laboratory-controlled experiments.

The outcomes from this research will inform future management strategies of flooded sites that will aid to protect public health.

References

[1] Yard et al., 2014. J. Environ. Sci. Health 49, 1236-1243.

[2] Hussain, 2019. Freshwater Microbiology 10, 393-420.

[3] Douterelo et al., 2009. Int. Biodeterior. Biodegradation 63, 6, 795-805.

## P305

# Automated analysis of genomic sequences facilitates high-throughput and comprehensive description of bacteria

Thomas Hitch[1], Thomas Riedel[2], Aharon Oren[3], Jörg Overmann[2], Trevor Lawley[4], Thomas Clavel[1]
[1]Functional Microbiome Research Group, RWTH University Hospital, Aachen, Germany. [2]Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany. [3]The Institute of Life Sciences, The Hebrew University of Jerusalem, The Edmond J. Safra Campus, Jerusalem, Israel. [4]Host-Microbiota Interactions Laboratory, Wellcome Sanger Institute, Hinxton, United Kingdom

## Abstract

Microbiome research is hampered by the large fraction of still unknown bacteria. However, many of these species have been isolated, yet lack a validly published name. The validation of names for novel bacteria requires that the uniqueness of those taxa is demonstrated and their properties are described. The accepted format for this are protologues, which are time-consuming to create. Hence, many research fields in microbiology and biotechnology will greatly benefit from new approaches that reduce the workload and harmonize the generation of protologues.

We have developed Protologger, a bioinformatic tool that automatically generates all the necessary readouts for writing a detailed protologue. By producing multiple taxonomic outputs, functional features and ecological analysis using the 16S rRNA gene and genome sequences from a single species, the time needed to gather the information for describing novel taxa is substantially reduced. The usefulness of Protologger was demonstrated by using three published isolate collections to describe 34 novel taxa, encompassing 17 novel species and 17 novel genera, including the automatic generation of ecologically and functionally relevant names. We also highlight the need to utilise multiple taxonomic delineation methods as while inconsistencies between each method occur, a combined approach provides robust placement. Protologger is open source; hence, all scripts and datasets are available, along with a webserver at www.protologger.de

## P306

# Inhibition of ammonia monooxygenase from ammonia oxidising archaea by linear and aromatic alkynes

Chloe Wright, Arne Schatteman, Andrew Crombie, Colin Murrell, Laura Lehtovirta-Morley
University of East Anglia, Norwich, United Kingdom

## Abstract

Nitrification is a key microbial process in the global nitrogen cycle and results in enormous losses of ammonium-based fertilisers and environmental pollution. Autotrophic archaeal and bacterial ammonia oxidisers (AOA and AOB, respectively) initiate nitrification through the oxidation of ammonia ($NH_3$) to hydroxylamine ($NH_2OH$), a reaction catalysed by ammonia monooxygenase (AMO). AMO belongs to the same copper-dependent monooxygenase family (CuMMO) as the particulate methane monooxygenase (pMMO) from methanotrophs. The bacterial AMO and pMMO co-oxidise a broad range of hydrocarbon substrates but little is known about the substrate range of the archaeal AMO. The aim of this study was to provide insights into the archaeal AMO by comparing the response of archaeal AMO, bacterial AMO and pMMO to inhibition by linear 1-alkynes ($C_2$-$C_8$) and the aromatic alkyne, phenylacetylene, in pure culture. To assess the inhibition of the different CuMMOs, $NH_3$-dependent $NO_2^-$ production was measured. Microorganisms included two phylogenetically distinct strains of AOA, "*Ca.* Nitrososocosmicus franklandus" and "*Ca.* Nitrosotalea sinensis", the AOB, *Nitrosomonas europaea*, and the methanotroph, *Methylococcus capsulatus* (Bath). As previously reported for other archaeal genera, reduced sensitivity to inhibition by larger alkynes (>$C_5$) was observed in AOA compared to AOB, suggesting that the archaeal AMO has a narrower hydrocarbon substrate range. The findings further validate the use of 1-octyne ($C_8$) to distinguish between the nitrifying activities of AOA and AOB in natural environments. Kinetic analysis revealed phenylacetylene inhibited "*Ca.* Nitrososocosmicus franklandus" and *N. europaea* AMO at different concentration ranges and by different mechanisms of inhibition, highlighting functional differences between the archaeal and bacterial AMO.

## P306

# Ecosystem engineering in Pseudomonas fluorescens SBW25 experimental microcosms

Robyn Shannon Jerdan, Andrew Spiers
Abertay University, Dundee, United Kingdom

## Abstract

Environmental modification through ecosystem engineering and niche creation (EE/NC) is commonly observed within ecological studies. This is demonstrated using diversifying *Pseudomonas fluorescens* SBW25 populations in static liquid microcosms, where $O_2$-uptake in the liquid column by initial wild-type colonists creates a high-$O_2$ ecological niche directly below the air-liquid (A-L) interface at the top of the liquid column. Here, Wrinkly Spreader (WS) adaptive mutants can better exploit this ecological opportunity through biofilm-formation. However, metabolic activity also utilises nutrients and releases secondary metabolites and waste products, which may have an additional EE/NC effect. In this research we demonstrate the effects of significant chemical changes that occur in King's B media through wild-type SBW25 metabolic activity. Aging media (cleared cell-free supernatant) could be differentiated by FTIR specta, pH changes and an increase in eDNA levels, and demonstrated a reduced ability to support further growth, suggesting a tragedy of the commons effect in which population growth progressively degrades the environment. WS mutants subjected to an aged environment showed altered biofilm-characteristics, and mutants evolved in one-day aged media could be phenotypically differentiated from those evolved in fresh media. However, these mutants demonstrate increased fitness in both an aged environment and in fresh media, suggesting adaption to the chemically-altered environment of aging static microcosms (a condition for NC). This research provides further insight into the ecological dynamics of static liquid microcosms, where EE/NC by basic biological processes can occur over a short time-period and significantly alter the selective pressures on adaptive lineages and their phenotypes.

## P307

# Polymycovirus-induced growth enhancement of *Beauveria bassiana* depends on carbon & nitrogen metabolism

Rebecca M. Diss[1], Charalampos Filippou[2], Robert H.A. Coutts[2], Ioly Kotta-Loizou[1]
[1]Imperial College London, London, United Kingdom. [2]University of Hertfordshire, Hatfield, United Kingdom

## Abstract

*Beauveria bassiana* is an entomopathogenic fungus used as a biocontrol agent against a range of insect pests. Despite the environmental advantages to biocontrol agents, chemical pesticides are often preferred because of their low cost and efficiency. Infection of *B. bassiana* strains EABb 92/11-Dm and ATHUM 4946 with double-stranded RNA viruses Beauveria bassiana polymycovirus (BbPmV)-1 and -3, respectively, increases their growth and virulence. Here, a time-course experiment comparing *B. bassiana* virus-free and virus-infected isogenic lines on different carbon (C) and nitrogen (N) sources revealed that virus infection interferes with host metabolism. Fungal conidiospores were inoculated onto variations on Czapek-Dox minimal medium, where the original C (sucrose) and N (sodium nitrate) sources were substituted with other C/N sources, and their radial growth was measured over the course of 18 days. When sucrose was replaced with fructose or maltose, the virus-mediated increase in growth was reversed in both EABb 92/11-Dm and ATHUM 4946 strains. Additionally, when sodium nitrate was replaced with sodium nitrite, potassium nitrate or ammonium nitrate, a reversal of the virus-mediated growth increase was also observed. These data suggest that BbPmV-1 and BbPmV-3 interfere with both carbohydrate metabolism and nitrogen assimilation. Identifying host processes targeted by these viruses gives us a better understanding of their effects on fungal growth and has the potential to be harnessed to increase the efficiency of *B. bassiana* biocontrol formulations.

## P308

# Maximising productivity and eliminating *Campylobacter* in broilers by manipulating stocking density using 'biosecurity cubes'.

Genevieve Greene[1,2], Leonard Koolman[1], Paul Whyte[2], Declan Bolton[1]
[1]Teagasc Food Research Centre, Dublin 15, Ireland. [2]University College Dublin, Dublin 4, Ireland

## Abstract

*Campylobacter* is the primary cause of foodborne bacterial gastroenteritis with approximately 250,000 EU cases reported annually. Broilers are the main source of human infection, with 38% of broiler carcasses in the EU contaminated. As such, poultry farms are considered the most effective control point, and enhanced biosecurity the optimum method to achieve this. Our research group previously designed a "biosecurity cube" which effectively prevented *Campylobacter* colonisation of a sub flock. Recently, we upscaled the biosecurity cube into a biosecurity framework while also observing improvements in broiler growth and wellbeing. Initial trials focused on identifying an optimum barrier material (cardboard, wire mesh, polyurethane film, or flyscreen mesh) to prevent bird to bird contact thus preventing *Campylobacter* colonisation. The experiment was then upscaled to a "biosecurity framework" of 8 biosecurity cubes stocked at varying densities (12, 14, 16, 18, 20 and 22 birds/$m^2$), with the main flock (20 birds/$m^2$) used as the control. Periodically, birds were randomly selected in each cube, weighed, examined, and the *Campylobacter* status of the birds monitored. The biosecurity framework prevented *Campylobacter* colonisation of test flocks over two separate rearing periods even when the surrounding flock tested positive after first thin. Additionally, test birds reached a target weight (2Kg) 3-6 days faster than the general flock stocked at the same density (20 birds/$m^2$). We conclude that biosecurity cubes present a potential solution to the *Campylobacter* problem, allowing for the production of *Campylobacter* free broilers, while also increasing productivity via optimised stocking densities.

**P309**

# Oxidases production and aflatoxin degradation during the interaction process of *Aspergillus flavus* and *Pleurotus ostreatus*

Yetzemany Huitrón-Contreras[1], Soley Nava-Galicia[2], Saraí Cruz-Pacheco[2], Martha Bibbins-Martínez[2]
[1]CIBA- INSTITUTO POLITÉCNICO NACIONAL, Tepetitla, Tlaxcala, Mexico. [2]CIBA-INSTITUTO POLITÉCNICO NACIONAL, Tepetitla, Tlaxcala, Mexico

## Abstract

Aflatoxin contamination caused by the fungal pathogen *Aspergillus flavus* is a significant threat to global health and food quality. The development of new strategies for eliminating aflatoxin contamination, such as enzyme treatments, is an important, viable, and commercially useful approach. The oxidases produced by the white rot fungus *Pleurotus ostreatus* are nonspecific enzymes capable of catalyzing the oxidation of many types of organic compounds, including aflatoxins.

The present research studied the metabolic response occurring in the interaction between *Pleurotus ostreatus* and *Aspergillus flavus* in order to ascertain the effect of said interaction on fungal growth, the production of aflatoxins and the enzymatic activity of oxidases. The present study also sought to define the possible participation of these enzymes in aflatoxin degradation.

The interaction between *P. ostreatus* and *A. flavus* was observed to affect both the growth of *A. flavus* and its production of aflatoxins, with the speed of said growth found to decrease by 22 %. An induction of aflatoxin production was observed on the ninth day of the interaction, reaching a maximum level by day 18 and then decreasing from days 23-30, while, for the control group, aflatoxins were detected on day 18 and increased in level until day 30 of the assay.

The interaction assay obtained maximum enzymatic activity values for the dye peroxidase enzyme (1466.85 UI/L) and manganese peroxidase (1246.33 UI/L). In the control group, the enzyme laccase presented maximum values of 1661.88 UI/L, while the activity of the remaining enzymes studied were much lower.

## P310

# An experimental evolution system to study pasteurization resistance in *Legionella pneumophila*

Jeffrey Liang[1], Sebastien P. Faucher[2]
[1]McGill University, Sanite-Anne-de-Bellevue, Canada. [2]McGill University, Sainte-Anne-de-Bellevue, Canada

## Abstract

Following its discovery in 1976, *Legionella pneumophila* has become a bacterial pathogen of high concern, particularly owing to its ability to colonize water distribution systems and thereby disseminate into vulnerable hospital populations. Though pasteurization is commonly used to flush a system of *L. pneumophila*, resident populations typically rebound after treatment ends. To study the possible mechanisms involved in the recolonization of hot water systems, we established an experimental system to re-capitulate the selective pressures of repeated incomplete pasteurization. Over 70 passages of this experimental evolution model, six independent lineages of *L. pneumophila* have been produced with substantial increases in their abilities to tolerate heat shock relative to their ancestor strain. To control for the artificial culture conditions of this model, six control lineages have been passaged in parallel without selection for pasteurization resistance. Short-read sequencing of isolates from the final populations has shown a collection of mutations accumulated over the experimental evolution process. Genes which were mutated in multiple treatment lineages include *htpG*, *rodA*, *clpB*, *clpX*, *dnaJ*, *dnaK*, and *nuoG*. All these mutations represent non-synonymous SNP's, except for one frameshift and one nonsense mutation in the *htpG* gene, which codes for the bacterial 90 kilodalton chaperone. Experimental findings support that *L. pneumophila* mutants with a deletion of *htpG* have an increased tolerance to heat shock. This model confirms that heritable genetic changes are involved in the increased pasteurization tolerance of *L. pneumophila* populations contaminating hot water systems.

## P311

# Surface Plasmon Resonance imaging (SPRi) based detection of *Legionella pneumophila* using aptamers in a titration assay

Mariam Saad, Francisco Castiello Flores, Sebastien Faucher, Maryam Tabrizian
McGill University, Montreal, Canada

## Abstract

Background: Water system closures and re-openings in response to the COVID pandemic shutdowns/lockdowns has led to an increase in the proliferation of *Legionella pneumophila* (*Lp*), a wide-spread pneumonia-causing, waterborne pathogen. Rapid and effective detection methods are urgently needed to improve water management strategies and prevent the spread of *Lp*. Aptamer-based biosensors can provide rapid, effective detection platforms. Aptamers are DNA/RNA molecular probes, analogous to antibodies, whose chemical composition, make them versatile as bioreceptors for sensor-based applications.

Methods: Aptamers were created against *Lp* using Cell-SELEX, an iterative process through which cell bound sequences are selected and amplified, while unbound sequences are discarded. Final pools have sequences with high affinity and specificity for *Lp*. Two aptamers, R10C1 and R10C5, were identified and characterized via microscopy and flow cytometry. R10C5 was optimized for use in a Surface Plasmon Resonance (SPRi) sensing platform to develop a titration assay, where the concentration of *Lp* was determined by quantifying the amount of unbound aptamers.

Results: The combination of aptamer titration assay with SPR enabled specific detection of *Lp* to an LOD of $10^{4.3}$ CFU/ml, without the use of labelling or signal amplification strategies. The SPR based assay also showed how different media-namely PBS, model tap water (Fraquil) and SSC, affects the ability of the aptamers to bind to *Lp*. Importantly, the assay shows minimal detection of *Pseudomonas*, a common inhabitant of water systems.

Conclusion: We developed a promising SPRi-based *Legionella pneumophila* detection platform by using *Legionella*-specific aptamers.

**P312**

# Phenotypic diversity of a clonal *Ralstonia solanacearum* pathogen lineage is explained by accessory genome variation

Evie Farnham[1], Martina Stoycheva[1], John Elphinstone[2], Daniel Jeffares[1], Ville Friman[1]
[1]University of York, York, United Kingdom. [2]FERA Science Ltd., York, United Kingdom

## Abstract

*Ralstonia solanacearum* is a plant pathogenic gram-negative bacterium capable of infecting several economically important crops such as potato and tomato. It can also persist in environmental reservoirs including soils, rivers and in asymptomatic wild hosts, causing disease outbreaks during pathogen spillover events when crossing agroecological interface. In the UK, *R. solanacearum* outbreaks originate from *Solanum dulcamara* wild hosts (woody nightshade) and river networks. To what extent selection in these natural environments drive *R. solanacearum* survival and life history evolution including virulence is unknown. To study this, we focused on a largely clonal *R. solanacearum* lineage inhabiting river networks across the UK consisting of a collection of 182 isolates spanning 30 years since the first outbreak in 1992. We first characterised strains phenotypically regarding 32 traits including resource catabolism, virulence and abiotic stress tolerance and then used microbial GWAS techniques to identify links between phenotypic traits and the presence of specific accessory genes. We found that isolates can be clustered into three phenotypic groups, which differed clearly regarding their resource specialism and stress tolerance. No effect of isolation location was found. However, isolates became more variable phenotypically along with time. While only few SNPs were found to vary among all isolates, the presence and absence of certain accessory genes, such as S-layer family protein, could be associated with phenotypic variation in terms of survival in stressful environments. Together, our findings suggest that a low number of accessory genes can cause high phenotypic variability within highly clonal bacterial lineage.

**P313**

# PROFILING ANTIMICROBIAL PEPTIDES FROM MYXOBACTERIA AS SUITABLE ANTIBACTERIAL AGENTS

Benita Arakal[1], Paul Livingstone[1], David Whitworth[2]
[1]Cardiff Metropolitan University, Cardiff, United Kingdom. [2]Aberystwyth University, Aberystwyth, United Kingdom

## Abstract

Bacteria that are recalcitrant to antibiotics have exhausted treatment options thus shifting focus on therapeutic substitutes of which antimicrobial peptides (AMPs) demand attention. Predatory bacteria suchlike myxobacteria are proven to produce antimicrobial secondary metabolites, although AMPs produced by them could signify bioactivity.

On hunting myxobacterial-AMPs, peptide sequences from eight complete genomes of myxobacteria accessible on NCBI database were extracted using an AMP-prediction tool. Among the eight, *Haliangium*, *Stigmatella* and *Sorangium* possessed maximum AMPs (>100). Further in-silico screening for antibacterial activity of myxobacterial-AMPs against five bacterial pathogens revealed that AMPs from *Haliangium*, *Stigmatella* and *Anaeromyxobacter* were effective against all five bacteria while AMPs from *Sorangium* were active against four bacteria and *Myxococcus spp*. against three bacteria. Protein-BLAST on all AMPs revealed several AMPs that generated 100% sequence similarity amongst other myxobacteria that could reflect on its evolutionary relationships. Probing these AMPS in determining their association with other proteins revealed linkages of some AMPs to proteins with lytic activity that could help decipher predatory mechanisms. Conducting pangenome analyses for *M.xanthus* and *C.coralloides* genomes procured from NCBI and AMP-inspecting its core and accessory genomes depicted an abundance of *C.coralloides*-AMPs shared between its core and accessory genomes while *M.xanthus*-AMPs were relatively low. On comparing the secondary metabolites and AMPs for all eight myxobacterial genomes, a higher proportion of secondary metabolites were observed than AMPs.

However, the exact magnitude of this preponderance of secondary metabolites needs wet-laboratory experiments to prove their functional capabilities. Scrutinizing myxobacterial-AMPs provides a novel dimension in combating antibiotic resistance.

## P314

# Soil acidification increases viral diversity and potentially changes soil ecological function

Erinne Stirling[1,2,3,4], Yiling Wang[1,2], X.F. Lv[5], Bin Ma[1,2,3]
[1]Institute of Soil and Water Resources and Environmental Science, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou, China. [2]Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Hangzhou, China. [3]Hanzhou Global Scientific and Technological Innovation Center, Zhejiang University, Hangzhou, China. [4]Acid Sulfate Soils Centre, School of Biological Sciences, The University of Adelaide, Adelaide, Australia. [5]Department of Environmental Engineering, China Jiliang University, Hangzhou, China

## Abstract

**Background:** Soil microbial organisms are well known to affect plant health and mediate plant responses to environmental stressors such as soil acidification. However, the response of soil viruses to soil acidification is largely unknown even though accelerated soil acidification is an issue that affects vast tracts of agricultural land.

**Methods:** We mined viral contigs from bulk soil metagenomic data that had been collected from a long term nitrogen fertiliser application field trial located on a Ferralic Cambisol. Viruses, virus-host relationships and auxiliary metabolic genes (AMGs) were considered against the background soil acidification gradient.

**Results:** There was a complete turnover in the vial community between low (pH<5.7) and high (pH>5.7) pH groups, with the acidic samples having greater viral diversity and abundance. Virus-host relationships were also strongly determined by soil pH; however, this was due to restrictions to viruses rather than their hosts. Viral AMGs were also detected at a higher rate in the acid samples, with genes for carbohydrate metabolism, lipid metabolism, and xenobiotic biodegradation and metabolism significantly negatively correlated with soil pH.

**Conclusion:** Soil acidification increased the relative abundance and community composition of lysogenic viruses in the soil microbial biomass and also changed the expression of AMGs, indicating that soil acidification can drive viruses to change soil microbial function.

## P315

# Airborne prokaryotic community of an urban environment in the UK

Hokyung Song, Nicholas Marsden, Jonathan Lloyd, Clare Robinson, Christopher Boothman, Ian Crawford, Martin Gallagher, Hugh Coe, Grant Allen, Michael Flynn
The University of Manchester, Manchester, United Kingdom

## Abstract

There is still a lack of studies on airborne microbial communities in urban outdoor environments compared with indoor environments. To address this information gap, we collected samples from an urban air quality supersite located in Manchester, UK. Samples were collected in the two different time periods: 1) November, 2019 and 2) February, 2020. We used Illumina MiSeq sequencing technique targeting the 16S rRNA genes of prokaryotes. The major taxonomic groups we found included close relatives to known *Saccharopolyspora, Methylobacterium*, and *Rubellimicrobium* species which have often been found in atmospheric samples from other studies. Chlorine concentration was significantly higher during the sampling period in February, 2020 compared with November, 2019, agreeing with the back trajectory of the winds coming from North-West marine environment. In contrast, iron, copper, and zinc concentrations were higher during the sampling period in November, 2019 reflecting stronger anthropogenic impacts. Interestingly, amongst the 120 most abundant operational taxonomic units, the samples collected in November, 2019 had significantly higher relative abundance of operational taxonomic units associated with marine environments, for example, operational taxonomic units belonging to SAR86 clade and Marinimicrobia compared with the samples collected in February, 2020. In contrast, the samples collected in February, 2020 had significantly higher relative abundance of operational taxonomic units affiliated with *Methylobacterium* species, which are known to be resistant to high metal concentrations. Using the high-throughput sequencing technique, we were able to characterize the airborne prokaryotic community of an urban environment in the UK and understand the factors influencing microbial community composition.

## P316

# Mining the soil microenvironment for bacteria capable of degrading waste plastic

Sophie A. Howard, Habteab Isaack Sbahtu, Ronan R. McCarthy
Brunel University London, Uxbridge, United Kingdom

## Abstract

Plastic waste is a global concerning issue and each year approximately 350 to 400 million tons of plastic are produced. Current plastic waste management is poor, expensive and harms the environment. It is estimated that by 2050, 12,000 Mt of plastic waste will be accumulated if current plastic waste approaches are not changed. This study focuses on identifying a microbial solution to the plastic waste problem by screening for plastic degrading microorganisms. Soil samples were collected from various environmental locations and *Pseudomonas* species were isolated with *Pseudomonas* isolation agar. Isolated *Pseudomonas* species were screened for capacity to degrade polycaprolactone (PCL) plastic, through a zone clearing assay. We identified 25 *Pseudomonas* species capable of producing clearance zones in this assay. This activity was further investigated by determining the capacity of these bacteria to grow in minimal media supplemented with PCL or linear low-density polyethene (LLDPE) as the only carbon source. PCL and LLDPE weight loss and $OD_{600}$ measurements demonstrated that a number of our isolates could utilize PCL or LLDPE as their sole carbon source. Further investigation of plastic degrading activity is underway, such as scanning electron microscopy and flow cell imaging. We are currently sequencing the genomes of these isolates and performing transposon mutagenesis to identify the specific enzymes involved in degrading plastic. The identification of bacteria that are capable of degrading plastic and the characterisation of the proteins involved is an important step towards developing a biotechnological tool to use against the growing plastic waste problem.

**P317**

# An imaging and modelling framework for studying bacterial chemotaxis in transparent soil

Daire Carroll[1,2], Nicola Holden[3], Miriam Gifford[1,4], Lionel Dupuy[5,6]
[1]University of Warwick, School of Life Science, Coventry, United Kingdom. [2]The James Hutton Institute, Dundee, United Kingdom. [3]SRUC, Northern Faculty, Aberdeen, United Kingdom. [4]University of Warwick, Warwick Integrative Synthetic Biology Centre, Coventry, United Kingdom. [5]Neiker, Department of Conservation of Natural Resources, Derio, Spain. [6]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

## Abstract

The ability of soil-borne bacteria to detect and colonise roots plays a pivotal role in determining plant health. Microorganisms respond to components of plant root exudate through chemotaxis: a directional movement in response to a concentration gradient. Experimental quantification of chemotaxis has traditionally taken place in homogenous liquid or gel medias. In these conditions, the impact of soil structure on bacterial activity is lost. Therefore, we have utilized an artificial substrate, transparent soil, inoculated with fluorescently tagged bacteria for live quantification of the response of bacteria to a chemoattractant in soil-like conditions. Through imaging, we tracked the movement of *Pseudomonas fluorescens* (SBW25) in the presence and absence of root exudates from lettuce (*Lactuca sativa*). We used various models to extract bacterial growth, dispersion, and chemotactic parameters from images. Finally, whole plant root assays were performed to access colonisation by *P. fluorescens*. Our approach allows identification and quantification of bacterial movement in structured media. These processes have previously been largely out of reach as they are hidden within soil. Our model is a step towards understanding how plant and bacterial traits combine in the soil to determine colonisation patterns. It can readily be coupled with models of growth and attachment on cells. This will be a valuable tool for the selection of microbes with beneficial plant-associated traits within the complex plant-microbiome environment.

## P318

# Visualisation of Extraluminal Bacterial Migration on Indwelling Urinary Catheters: Development of a Novel In Vitro Urethral Migration Model

Yvonne J Cortese[1], Joanne Fayne[2], Declan Devine[1], Andrew Fogarty[1]
[1]Athlone Institue of Technology, Athlone, Ireland. [2]Teleflex, Athlone, Ireland

## Abstract

Catheter-Associated Urinary Tract Infections (CAUTIs) are one of the most common healthcare-associated infections in the world and can lead to increased patient morbidity and mortality. Regarding indwelling urinary catheters, infection is regularly caused by bacterial migration along the intra- and extraluminal surfaces of the *in situ* catheter. Extraluminal migration allows bacteria to ascend, by either self-motility or capillary action, from the urethral meatus along the catheter surface to the bladder. It was the aim of this study to develop an *in vitro* model to study and visualise this extraluminal migration, and to provide a tool for the development of novel catheters and coatings to prevent extraluminal bacterial migration. The *in vitro* urethral migration model consists of a preformed channel within an agar-based matrix. The urethral model utilises chromogenic agar to visualise bacterial migration over an extended incubation period to mimic longterm catheterisation. During the early stages of model development, bacterial swarming and condensation presented a challenge which was addressed by a redesign of the urethra model mould and incubation conditions. Inadequate oxygenation within the *in vitro* urethra was addressed by modification of the urethra channel within the model. Preliminary tests have demonstrated reproducible results. The *in vitro* urethral migration model is currently under validation. The bacterial migration urethral model aims provide a robust tool to aid in the development of future urological devices focused toward preventing CAUTIs and improving clinical outcomes for patients.

**P319**

# Meteorites: beneficial or toxic for life on Early Earth? Growth of an anaerobic microbial community on a carbonaceous chondrite

Annemiek C. Waajen[1], R. Prescott[2,3], Charles S. Cockell[1]
[1]UK Centre for Astrobiology, University of Edinburgh, Edinburgh, United Kingdom. [2]Department of Environmental Health Sciences, University of South Carolina, Columbia SC, USA. [3]School of Life Sciences, University of Hawai'i at Mānoa, Honolulu, HI, USA

## Abstract

Meteoritic material accumulated on the surface of the anoxic Early Earth during the Late Heavy Bombardment around 4.0 Gya. These meteorites may have provided the Earth with extra-terrestrial nutrients and energy sources for early life. How could the presence of meteorites have affected the origin and evolution of early life on Earth? And what is the influence of geothermal activity on the Earth's surface? This research investigates the growth of an anaerobic microbial community from pond sediment on non-pyrolyzed (pristine) or pyrolyzed (heat-treated) carbonaceous chondrite 'Cold Bokkeveld'. A microbial community was grown anaerobically in batch cultures containing a liquid environment and powdered non-pyrolyzed or pyrolyzed Cold Bokkeveld. Cell concentrations were measured by Colony-Forming Units on agar plates. The community composition in the presence of non-pyrolyzed meteorite was determined by 16S rRNA amplicon sequencing. Non-pyrolyzed Cold Bokkeveld supported the growth of a stable, anaerobic community containing mainly the Deltaproteobacteria *Geobacteraceae* and *Desulfuromonadaceae*. Members of these families are known to use elemental sulfur and ferric iron as electron acceptors, and organic compounds as electron donors. Pyrolyzed Cold Bokkeveld however, was inhibitory to the growth of the microbial community. These results show that carbonaceous chondrites can host an anaerobic microbial community, but that pyrolysis, e.g. by geothermal activity, can inhibit microbial growth and potentially toxify the material. This indicates that extraterrestrial meteoritic material and the environment on Early Earth could have shaped the nature of early microbial ecosystems by enhancing growth of microorganisms with metabolic capabilities favored in the presence of this material.

**P321**

# NATURAL AND FUNCTIONAL POLYHYDROXYALKANOATE OLIGOMERS DERIVED FROM BREWERY WASTE

Anabel Ekere[1], Liam Cowburn[2], Fidelina Tchuenbou-Magaia[1], Wanda Sikorska[3], Magdalena Zięba[3], Grazyna Adamus[3], Iza Radecka[1], Marek Kowalczuk[3]
[1]University of Wolverhampton, Wolverhampton, United Kingdom. [2]Banks Brewery, Wolverhampton, United Kingdom. [3]Centre of Polymer and Carbon Materials, Polish Academy of Sciences, Zabre, Poland

## Abstract

High molecular weight Polyhydroxyalkanaotes (PHAs) are attractive biomaterial in research owing to their desirable properties. Nevertheless, its application in other advanced areas of importance is limited due to its poor mechanical properties and hydrophobic nature [1]. Alternatively, PHA oligomers are low molecular weight PHAs that possess a controlled molecular weight distribution and functional end groups which can be further used as building blocks for the synthesis of new polymers with improved functionalities and properties [2]. Brewery waste containing yeast biomass was investigated in this study for its ability to intracellularly accumulate natural PHA oligomers. Brewery liquidified waste was centrifuged and lyophilized for 48 h. The dried yeast biomass was transferred into extraction thimbles to have PHA oligomers extracted by soxhlet extraction with chloroform for 48 h. Using a rotary evaporator, the resulting chloroform/PHA oligomer mixture was concentrated and precipitated afterwards to obtain pure PHA oligomers. The obtained PHA oligomer was characterized using Fourier transform infrared spectroscopy (FTIR), Nuclear magnetic resonance spectroscopy (NMR) and its yield was also determined. The yield for the PHA oligomer obtained was determined to be 1.5 % w/w (±). FTIR spectra showed signals characteristic of polyhydroxybutyrate (PHB) and NMR spectrum further confirmed the presence of PHB units in the oligomer obtained in this study. These biodegradable oligomers, through various chemical modifications can further permit the preparation of bioactive oligomers with attractive properties for novel, tailored and high valued applications, particularly in medicine, agrochemistry and cosmetology [3,4,5].

## P322

# Aquatic fungi-bacteria interaction on particulate organic matter – friends or foes?

Cordelia Roberts[1,2], Ro Allen[1], Kimberley Bird[1], Michael Cunliffe[1,2]
[1]Marine Biological Association, Plymouth, United Kingdom. [2]University of Plymouth, Plymouth, United Kingdom

## Abstract

Microbial colonisation and degradation of particulate organic matter (POM) are important processes that influence the structure and function of aquatic ecosystems. Chitin is a widespread polymer component of aquatic POM and important source of carbon and nitrogen. Bacteria and fungi readily colonise chitin and can degrade the polymer in aquatic ecosystems yet reports on the interactions between these major taxa are limited. We have been studying fungi-bacteria interactions associated with chitin-based POM in both controlled laboratory experiments and in the field. Using chitin microbeads and a model chitin degrading chytrid fungi, we explored the impact of fungi on POM-associated bacteria. We show that the presence of fungi can alter the structure and function of concomitant bacterial communities, with differing responses across life stages. Analogous field-based experiments with chitin microbeads incubated with seawater collected from the North East Atlantic Ocean have been performed to determine if similar interactions take place with natural POC-associated fungi. We propose fungi can act as ecosystem facilitators through saprotrophic feeding, providing 'public goods' which modify bacterial POM colonisation, in addition to promoting degradation in attached bacterial communities. These studies suggests that fungi have complex ecological roles in aquatic POM degradation not previously considered, including the regulation of bacterial community development, functioning and subsequent biogeochemical cycling.

## P323

# Host mediated microbiome selection to study the changes in the nutrient cycling, root exudation and microbial population in the rhizosphere of soybean genotypes.

Deepa Manikkath Haridas
University of Reading, Reading, United Kingdom

### Abstract

The study was conducted with an aim to understand the changes in soil enzyme activity in the plant rhizosphere during host mediated selection of rhizosphere microbiome. Soil samples were collected from three different locations on a farm in the Reading area, UK. Two popular genotypes of Soybean (*Glycine max*) were chosen for the study, Kenchen and Siverka. Initial microbiome inoculum was prepared by mixing field soil with sterilized water and this was added to the autoclaved coir: sand mix. After four weeks of plant growth, plants were uprooted from the soil, rhizosphere soil collected for soil enzyme estimation and NGS analysis. Root exudates were collected and stored in freezer for analysis.

Growth of plants in legume soils was significantly higher. The plants in legume soil produced nodules which confirmed the presence of rhizobia in these soils. Independent analysis within each soil type showed that, the interaction between soil type and genotype is very significant with respect to enzyme activity. The presence of microbiome along with plants had a significant effect on enzymatic activity. GCMS analysis of root exudates from plants in each soil showed variations in individual chromatograms. The difference in enzyme activity with plant growth shows that there are changes taking place in the soil. Further in this study, NGS analysis of rhizosphere soil will shed more light into the changes happening in the soil microbiome and if the changes are related to the enzymatic changes and plant root exudation.

## P325

# Intraspecies-Resolution Metabarcoding: automated primer design and a plant pathology case study

Leighton Pritchard[1,2], Sonia Humphris[2], Emma Campbell[2], Lauren Watts[2], Ed Haynes[3], Hollie Pufal[3], Greig Cahill[4], Triona Davey[4], John Elphinstone[3], Ian Toth[2]
[1]University of Strathclyde, Glasgow, United Kingdom. [2]The James Hutton Institute, Dundee, United Kingdom. [3]Fera, York, United Kingdom. [4]SASA, Edinburgh, United Kingdom

## Abstract

We present results demonstrating species-specificity and sub-species resolution by novel, automatically-designed metabarcoding primers for environmental DNA analysis.

Conventional metabarcoding remains a cornerstone of rapid, high-throughput environmental DNA (eDNA) community analysis and biodiversity assessment. Standard barcodes such as 16S (prokaryotes) and ITS1 (fungi/oomycetes) have been instrumental in identifying the complex composition of communities using total eDNA. However, standard barcodes have limitations in terms of resolution and quantitation and, though genus-level identification can be reliable, species-level identification is often not possible.

To overcome the limitations of resolution, we implemented extensions to the diagnostic primer design tool pdp (https://github.com/widdowquinn/find_differential_primers) that enable automated design of metabarcoding markers and corresponding primers that are (i) specific to a prescribed taxon at species level and (ii) capable of discriminating between members of the same species. This allows for rapid, high-throughput measurement of diversity below species level for a target organism.

We aimed to survey geographical distribution and pathogen transfer of the widespread plant pathogenic bacterium *Pectobacterium atrosepticum* (*Pba*). This organism has considerable sub-species taxonomic structure identifiable using MLST and with whole-genome methods, but which is not accessible using standard barcodes. We designed metabarcoding primers (202bp) specific to *Pba* using `pdp`, and established that these have resolution comparable to eight-gene MLST, revealing sub species-level diversity within single fields, and on the same individual plant host.

## P326

# Investigation of the anaerobic microbiota of beef carcass and rump steak cuts from five meat plants using high- throughput sequencing

Eden Esteves[1,2,3], Tanushree Gupta[4], Paul Whyte[2], John Mills[4], Gale Brightwell[4,5], Declan Bolton[1]
[1]TEAGASC, Dublin, Ireland. [2]University College Dublin, Dublin, Ireland. [3]Ag Research Limited, Palmerston North, New Zealand. [4]AgResearch Limited, Palmerston North, New Zealand. [5]Massey University, Palmerston North, New Zealand

## Abstract

Vacuum packaging of beef extends shelf-life by preventing the growth of aerobic bacteria. However, anaerobic bacteria can still cause spoilage. The presence of anaerobic microflora on fresh beef carcass and rump steaks, which may contribute to meat spoilage, was explored. A total of 120 carcass and 120 rump steak swabs were collected in five meat plants. Swab samples were anaerobically enriched and incubated at 4°C for 3 weeks. This was followed by DNA extraction and 16S rRNA amplicon sequencing using the Illumina MiSeq™, with subsequent bioinformatics analysis. The enriched microbiota of the samples was classified and grouped into 149 operational taxonomic units (OTUs). Both sample types consisted mainly of *Carnobacterium,* with an average relative abundance of 28.4% and 32.8% in beef carcasses and beef rump steaks, respectively. This was followed by *Streptococcus*, *Serratia*, *Lactococcus*, *Enterococcus*, *Escherichia-Shigella*, *Raoultella* and *Aeromonas* ranging from 1.5-20% and 0.1-29.8% in enriched carcasses and rump steak swabs, respectively. *Trichococcus*, *Bacteroides*, *Dysgomonas*, *Providencia*, *Paraclostridium* and *Proteus* were also present ranging from 0-0.8% on carcass and 0-1.8% on rump steak swabs, respectively. Alpha and Beta diversity measurements showed limited diversity between the two sample types, but some differences between samples from the beef plants investigated were evident. This may suggest that specific meat plants could be possible drivers of types of spoilage bacteria.

## P327

# Investigation of the functional and evolutionary links between pathways of xenobiotic and secondary metabolism in bacteria

Evanthia Kontomina[1], Vasiliki Garefalaki[1], Konstantina Fylaktakidou[2], Sotiria Boukouvala[1]
[1]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece. [2]Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece

### Abstract

Microbial secondary metabolites (SMs) are generated via the coordinated action of enzymes produced by biosynthetic gene clusters (BGCs). Microorganisms often produce SMs to chemically attack competitors, so microbial interaction is considered important for the activation of BGCs that would normally remain dormant. We are exploring the biosynthetic potential of 85 taxonomically characterised bacterial isolates from environmental or clinical specimens, by growing them in binary co-cultures. The screening of more than 3000 combinations has so far revealed several cases of antagonistic interactions, likely mediated by SMs with antimicrobial activity. In order to identify those compounds, the extracts of selected co-cultures of interest are being chemically analysed, guided by computational genome mining for BGCs. Furthermore, our *in silico* analysis has found arylamine *N*-acetyltransferases (NATs), an enzymatic family of particular interest to our lab, in various types of bacterial BGCs, where they are predicted to serve different functions. Bacterial NATs are better known as xenobiotic metabolizing enzymes active against harmful environmental pollutants. Screening of our entire bacterial collection against toxic arylamines demonstrated variable xenobiotic tolerance among isolates, with NAT-mediated *N*-acetylation often evident. As bacterial NAT homologues were demonstrated to participate in both SM biosynthesis and xenobiotic detoxification, we have consequently embarked onto comprehensive investigation of their functional divergence and phylogeny, helping trace back the postulated evolutionary links between pathways of secondary and xenobiotic metabolism in microorganisms. [Research co-financed by Greece and the European Union (ESF) through Operational Programme "Human Resources Development, Education and Lifelong Learning 2014-2020" in the context of project MIS 5050543.]

## P328

# Microbial community of MX80 bentonite and their interaction with iron

Katie Gilmour, Colin Davie, Neil Gray
Newcastle University, Newcastle-Upon-Tyne, United Kingdom

### Abstract

MX80 bentonite has been selected as the buffer and backfill in a proposed method of long-term deep geological storage of nuclear waste. Extensive studies have been carried out on the geomechanical properties of MX80; however, it is not clear what effect microbes will have on its ability to function as an effective barrier. Specifically, in the UK, as carbon steel waste canisters will contribute iron oxides and rust products to the immediate environment, iron-reducing bacteria are of interest. Iron-reducing bacteria can reduce structural or external Fe (III) to Fe (II) and some species are adapted to high temperatures and low water availability, in keeping with conditions within the waste repository. Indigenous iron-interacting bacteria have been identified in compacted MX80 and microbially-influenced iron-reduction was observed in groundwater salinity up to 0.45M NaCl. Experiments investigating gas production, and silica-solubilising abilities of this community were carried out. Further experiments in pressurised test cells investigated microbial activities at the clay / steel interface. Significant increases in hydrogen production were observed when microbes were present, and biogenically influenced changes in structure and appearance of MX80 were seen in all experiments. Additionally, silica release occurred, likely coupled to metal / microbe interactions. Corrosion products differed depending on microbial presence following incubation in test cells. Biogenic transformation of clay minerals through iron reduction or release of silica to groundwater could significantly impact the geomechanical properties of MX80, as indicated by observed changes in clay plasticity, and ultimately this could affect the behavior of the material as a barrier.

## P329

# Searching for new microbial enzymes for plastic polymer degradation: Polyurethanes and Lycra

Julia Linke[1,2], John Ward[1,2]
[1]University College London (UCL), London, United Kingdom. [2]Plastic Waste Innovation Hub, London, United Kingdom

### Abstract

New enzymes and biocatalysis could provide routes to help limit global plastic pollution, as suggested by the example of PETases for the PET degradation. Experiments were conducted to screen soil microorganisms for enzymes able to break down the plastic polymer Lycra, which contains polyurethane linkages and could be utilized as a carbon and nitrogen source.

The methodology is based on a plate assay, which compared the growth of bacteria at 50°C and 65°C on minimal media and minimal media with a top layer, that contained DMF-dissolved Lycra. The medium provided the main nutrients required for bacterial growth, with Lycra being the only substantial carbon source. Promising candidates were identified as bacterial colonies with visibly enhanced growth on the Lycra-enriched minimal medium plate. The selected soil microorganisms were then tested with a liquid culture assay, where the strain was cultivated at 50°C or 65°C in liquid broth medium, that contained dissolved Lycra. The liquid culture samples were collected at 4 time points between 0-48h, and the aliquots tested on HPLC for detection of released Lycra monomers.

Around 50 different bacterial strains from 6 various soil and compost sources were tested with the described plate screening method and 7 bacterial thermophilic species were detected, that showed visibly enhanced growth on the Lycra-enriched plates. These results could support the development of Lycra enzymatic degradation. New enzymes could be engineered, optimized and yield a sustainable solution for the textile industry, which would not require toxic chemicals or contribute to landfill.

**P332**

# Recycling-derived Phosphorus Fertilizers as a Sustainable Alternative to Triple Superphosphate Fertilizers

Lea Deinert[1], Israel Ikoyi[2], Achim Schmalenberger[1]
[1]Department of Biological Sciences, Faculty of Science and Engineering, University of Limerick, Limerick, Ireland. [2]Soil and Environmental Microbiology, Environmental Research Centre, Teagasc, Johnstown Castle, Wexford, Ireland

## Abstract

Microbial activity is adamant for the nutrient cycling in soil. Generally, mineral phosphorus (P) fertilizer is applied to soil to improve plant growth, however, significant amounts are immobilized quickly. Mineral fertilizer can cause soil degradation, affecting the microbial community. Alternative, recycling-derived fertilizers (RDFs) need to be evaluated as suitable replacement for finite mineral P fertilizer. The impact of four RDFs (two ashes, two struvites) on the soil microbiome in comparison with a P-free control and triple superphosphate (TSP) as mineral fertilizer was investigated in a pot trial and a subsequent microcosm trial (subset of samples). For both experiments, perennial ryegrass was cultivated for 54 days. The pot trial was conducted at P fertilization rates of 20 and 60 kg P $ha^{-1}$ in quadruplicates. After the pot harvest, the bulk soil was stored until the microcosm trial was conducted, using the control, TSP and the two ashes at 60 kg P $ha^{-1}$ in six replicates. Struvites displayed highest P bioavailability at high P application rates in the pot trial, yielding higher biomass on average. Furthermore, P solubilization from tri-calcium phosphate was enhanced in the RDFs treatments, while the TSP treatments were negatively affected. For the microcosm trial, most probable number (MPN) analysis showed that phytate-utilizing bacterial abundance was significantly increased in one of the ashes. Non-metric multidimensional scaling (NMDS) analysis of *phoD* illumina sequencing data showed significant separation between all treatments of the microcosm trial. Understanding the impact of RDF application on the soil P cycle is vital to sustainable agriculture.

## P333

# Shiga toxin-producing *E. coli* (STEC) isolated from wild mammals in Portugal

Diana Dias[1], Sávio Costa[2], Rafael Baraúna[2], Carlos Fonseca[3], Tânia Caetano[1], Sónia Mendo[1]
[1]CESAM and Department of Biology, University of Aveiro, Campus de Santiago, 3810-193, Aveiro, Portugal. [2]Centro de Genômica e Biologia de Sistemas, Universidade Federal do Pará, Belém, Brazil. [3]ForestWISE - Collaborative Laboratory for Integrated Forest & Fire Management, Quinta de Prados, 5001-801, Vila Real, Portugal

## Abstract

### Background

Zoonoses are diseases common to humans and animals (livestock, wildlife, and pets). In 2018 about 360 000 zoonoses were reported in European Union. Shiga toxin-producing *Escherichia* coli (STEC) infections were among the most reported causes of these zoonotic diseases.

### Methods

Faecal samples of mammal species (n=286) with distinct phenology (wild boar, red deer, otter, and red fox) were collected in Portugal. After the initial processing, the presence of STEC was screened by PCR, and suspicious samples were platted on CHROMagar STEC. STEC positive isolates were tested for antibiotic susceptibility. The phylogenetic relationship of STEC strains was evaluated by PFGE. Of these, 20 representative strains were selected for whole genome sequencing with the Illumina NovaSeq 6000 system. For the assembly, annotation and genome characterization, multiple web-based bioinformatic tools were employed.

### Results

Cultivable STEC (n=52) were recovered from 17% (n=49) of the samples collected from the four mammals. All the isolates were non-O157:H7 STEC encoding *stx1* (n=2; 4%) and/or *stx2* genes (n=51; 98%). Only one strain (2%) of red fox was resistant to ceftazidime, aztreonam and nalidixic acid. The 20 strains that were sequenced belong mainly to serotype O27:H30 (n=15), followed by O146:H28 (n=2), O146:H21 (n=1), O178:H19 (n=1) and O103:H2 (n=1). In addition to *stx*, all strains encode several virulence factors, mainly toxins, adhesins, fimbrae, secretion systems, among others. Additionally, several pathogenicity islands have been predicted for these strains.

### Conclusions

Our results show that wild animals are reservoirs of STEC, potentially pathogenic to humans.

## P334

# The UK Crop Microbiome CryoBank

Matthew Ryan[1], Tim Mauchline[2], Nicola Holden[3,4], Susan Jones[4], Jacob Malone[5,6]
[1]CABI, Egham, United Kingdom. [2]Rothamsted, Harpenden, United Kingdom. [3]SRUC, Aberdeen, United Kingdom. [4]James Hutton Institute, Dundee, United Kingdom. [5]John Innes Centre, Norwich, United Kingdom. [6]Univeristy of East Anglia, Norwich, United Kingdom

## Abstract

The UK Crop Microbiome CryoBank is a BBSRC-funded Bioinformatics and Biological Resource of cryopreserved and characterised crop microbiomes that aims to underpin national and international crop research. It will comprise material derived from six major UK crops: (barley, oats, oil seed rape, potato, sugar beet and wheat) grown in three different soil types. The project aligns with strategic priorities in agriculture and food security, driven by the needs of the academic and commercial agritech sector. Over the next five years, the project will generate bio-banked materials and bioinformatic tools to provide:

1. A cryopreserved resource of characterised material from crop microbiomes, consisting of culturable isolates and 'whole' microbiome samples. Samples will be available to the user community and will be dynamically linked to genomic data.
2. Robust methodologies for collection and storage of intact microbial communities in environmental samples and extracts of total DNA.
3. Advanced cryopreservation regimes to provide enhanced capability to sustainably maintain the resource in a genotypically and phenotypically stable state.
4. Genomic characterisation of the samples for assessing microbial diversity, from whole community taxonomies to individual isolate genomes
5. Aim to support discovery of new biological based products from PGPR isolation and synthetic community construction.
6. A validated sequence resources database, 'AgMicrobiome Base' linked to EBI, including model organisms and novel product outputs.

The resource will enable a more complete understanding of microbial community interactions in an agriculturally relevant context, for plant host-microbe and microbial community interactions.

## P335

# Isoprene monooxygenase: A missing link in the global isoprene cycle

Leanne Sims, Colin Lockwood, Andrew Crombie, Nick Le Brun, Colin Murrell
University of East Anglia, Norwich, United Kingdom

### Abstract

The ability to utilise isoprene is dependent upon a multistep pathway. The initial step in isoprene metabolism is oxidation of isoprene to epoxy-isoprene, catalysed by a four-component soluble di-iron monooxygenase (SDIMO), isoprene monooxygenase (IsoMO). IsoMO is a six protein complex comprising an oxygenase containing the di-iron active site (IsoABE), a Rieske-type ferredoxin (IsoC), NADH reductase (IsoF) and a coupling/effector protein (IsoD), homologous to the soluble methane monooxygenase and alkene/aromatic monooxygenases. We have demonstrated IsoMO activity with a range of alkene and aromatic substrates and explored the enzyme kinetics of IsoMO in whole cells.

We describe the purification of homologously expressed IsoMO proteins from a *Rhodococcus* sp. AD45 strain lacking the isoprene metabolic gene cluster, and characterisation of the Rieske-type ferredoxin component. We can reconstitute active IsoMO to enable biochemical and biophysical characterisation of the complete enzyme complex and the individual components of IsoMO. The knowledge gained allows further insights into the enzymatic basis for isoprene degradation in the environment.

## P336

# $Pb^{2+}$ removal from simulated wastewater by *Sporobolomyces* sp. cells immobilized on the fibrous network of *Careca papaya*

Chukwudi Onwosi, Daberechi Mela, Chukwudi Anyanwu
University of Nigeria, Nsukka, Nigeria

## Abstract

Heavy metals, with varying levels of toxicity to man and plants, are constantly introduced into the environment due to various anthropogenic activities. The use of microbial biomass is a reliable and environmentally friendly approach for heavy metal removal from contaminated soil and water. The objectives of this study are to assess the effectiveness of fungi isolated from waste battery dumpsite in the removal of lead ($Pb^{2+}$) from aqueous solution and to optimize the biosorption process using relevant models. The strain, identified as *Sporobolomyces* sp. and immobilized on *Careca papaya* fibrous network, was applied in bioremediation of $Pb^{2+}$ in an aqueous medium. The central composite design (CCD) was used to optimize the biosorption process, with three factors- pH, initial metal concentration, and biosorbent dose. The ability of the isolates to remove $Pb^{2+}$ from aqueous solution was evaluated using isotherm (Langmuir and Freundlich), kinetics (pseudo-first and -second-order), and the thermodynamics ($\Delta G^o$). The CCD revealed that the absorptive capacity (Qe = 81.9) can be achieved by combining the following parameters: pH = 4.5; initial $Pb^{2+}$ concentration= 141.9mg $L^{-1}$ and biosorbent dose = 0.2 g. The biosorption of $Pb^{2+}$ was estimated using Langmuir isotherm ($q_{max}$ = 144.24; $K_L$= 0.04; $R^2$ = 0.962) and was thermodynamically feasible ($\Delta G^o$ = +79.69 $Jmol^{-1}$) at high temperature. The biosorption process followed a pseudo-second-order path. In conclusion, the isolate from the waste battery dumpsite has the potential for $Pb^{2+}$ removal from wastewater.

## P338

# Biotransformation of phthalate plasticisers and bisphenol A by marine and freshwater fungi.

Andrew Cowan[1], Lena Carstens[2], Bettina Seiwert[3], Dietmar Schlosser[3]
[1]Solent University, Southampton, United Kingdom. [2]University of Duisburg-Essen, Duisburg, Germany.
[3]Helmholtz Centre for Environmental Research, Leipzig, Germany

### Abstract

Phthalate esters (PEs) are environmentally ubiquitous micropollutants that are used as plasticizers and additives in diverse consumer products. Considerable concern relates to their reported xenoestrogenicity and the microbial-based attenuation of environmental PE concentrations is of interest to combat harmful downstream effects. Fungal PE catabolism has received less attention than that by bacteria, and particularly marine fungal species remain largely overlooked in this respect. We have compared the biocatalytic and biosorptive removal rates of di-n-butyl phthalate (DBP) and diethyl phthalate (DEP), chosen as two environmentally prominent PE representatives (exhibiting differing structures and hydrophobicities), by marine- and freshwater fungal strains. Bisphenol A, both an extensively used plastic additive and prominent environmental xenoestrogen, was included as a reference compound due to its previously well-documented fungal degradation. Partial pathways for DBP metabolization by these ecophysiologically diverse ascomycetes were proposed with the help of UPLC-QTOF-MS analysis. Species-specific biochemical reaction steps contributing to DBP metabolism were also observed. The involved reactions include initial cytochrome P450-dependent monohydroxylations of DBP with subsequent further oxidation of related metabolites, de-esterification via either hydrolytic cleavage or cytochrome P450-dependent oxidative O-dealkylation, transesterification, and demethylation steps - finally yielding phthalic acid as a central intermediate in all pathways. Beyond previous research into fungal PE metabolism which emphasises hydrolytic de-esterification as the primary catabolic step, a prominent role of cytochrome P450 monooxygenase-catalysed reactions is established.

**P339**

# *In silico* identification of biofilm-important genes and proteins in pathogenic *Shigella* spp.

Connor E. Webb, Georgios Efthimiou
University of Hull, Hull, United Kingdom

## Abstract

Several *Shigella* species, such as *S. flexneri*, *S. sonnei* and *S. dysenteriae* are the causative agents of serious foodborne illness, therefore they are considered a major safety concern for the food industry. Formation of biofilms on food processing equipment and surfaces, makes these pathogens tolerant to detergents and difficult to eradicate, increasing the risk of spread via contamination of food products. This project aimed to use free, publicly accessible bioinformatic tools for identifying biofilm-important genes and proteins in pathogenic *Shigella* spp. in a remote capacity.

This study employed several genomic and proteomic tools (BLASTn (Megablast), BLASTp, STRING and the Artemis suite) together with various online databases (BioCyc, KEGG, GenBank, NCBI Nucleotide, UniProt) to identify key genes that are present in high-biofilm forming strains, but absent in low-biofilm formers.

Two genes (*agn and IsrR*) were found to yield no significant sequence matches in low-biofilm formers (*S. sonnei* and *S. dysenteriae*), with *S. sonnei* strains additionally demonstrating no significant sequence matches in three other genes (*fimA, fimC* and *elfC*). Protein homologues were present, although similarity matches were low (<44%) in low-biofilm formers, with high-biofilm formers (*S. flexneri*) sharing significant similarities both in gene and protein sequences (>89.2% and >88.1%, respectively). STRING networks based on these protein homologues were also constructed.

Shedding light on these complex networks will help the identification of novel genes and protein interactions in *Shigella* spp., using accessible, publicly available bioinformatics, ultimately allowing for *in silico* screening of pathogenic strains and the development of new biofilm eradication approaches.

## P340

# Increasing production of the antibiotic-abyssomicin C in *Micromonospora maris*

Yanhua Zhong, Jem Stach, Michael Hall
Newcastle University, Newcastle upon Tyne, United Kingdom

## Abstract

Abyssomicin C is a polyketide antibiotic produced by *Micromonospora maris* AB-18-032. Previous work on abyssomicin C indicated that it inhibits the growth of infectious pathogens such as Methicillin-resistant *S. aureus* (MRSA) and Vancomycin-resistant *S. aureus* (VRSA). It does this by suppressing *para*-aminobenzoic acid (*p*ABA) synthesis, which is required for folic acid biosynthesis in bacteria. This makes abyssomicin C an appealing antibiotic drug, as it is specific only to bacteria. However, its yield in chemical synthesis (4 %) and biosynthesis (60.0 mg/L) are low.

Ribosome engineering, through the selection of streptomycin-resistant, rifampin-resistant and gentamicin-resistant mutants, of *M. maris* may result in strains with a higher titre of production. Six mutants we were interested in, were selected from bioassay and HPLC. Of these mutants, four of them are able to produce a higher titre of abyssomicin C compared with wild type *M. maris*. The other two strains were detected to produce the other secondary metabolite. After that, the whole genome of these six mutants were sequenced and the mutant genes were identified.

**P341**

# Identifying long-term colonization factors of *Vibrio cholerae* O1 El Tor in the zebrafish natural host model

Madison G. Walton[1], Geoffrey B. Severin[2], Christopher R. Rhoades[2], Christopher M. Waters[2], Jeffrey H. Withey[1]
[1]Wayne State University School of Medicine, Detroit, MI, USA. [2]Michigan State University, East Lansing, MI, USA

## Abstract

The human disease cholera, marked by acute, voluminous watery diarrhea, is caused by the gram-negative, aquatic bacterium *Vibrio cholerae*. All seven cholera pandemics since 1817 were identified as being caused by just 2 of over 155 known *V. cholerae* serogroups: O1 and O139. The O1 serogroup is divided into two biotypes: classical and El Tor. Classical biotype is associated with pandemics 1 through 6, but the El Tor biotype has since displaced classical as the causative agent of the ongoing 7$^{th}$ cholera pandemic over the past 60 years. The El Tor genome resembles that of classical but has acquired two unique pathogenicity islands known as Vibrio Seventh Pandemic (VSP) -1 and -2. El Tor biotype has been associated with prolonged colonization, infection, and disease both in humans and in the zebrafish natural host model. The zebrafish model allows for complete observation of *Vibrio cholerae* infection in a system undisrupted by antibiotic use or immune suppression. El Tor strains colonize the zebrafish intestine for up to 10 days longer than classical strains. Preliminary studies demonstrate VSP-2 is required to observe this phenotype, but VSP-1 is not. By creating targeted regional knockouts of the VSP-2 island, the specific gene(s) essential for enabling prolonged colonization will be identified and applied to the understanding of how El Tor interacts with a natural host. By identifying the genes used by El Tor to colonize a natural host for prolonged periods, we gain insight into how this pathogen may persist in the environment and perpetuate disease.

**P342**

# The von Willebrand Binding Protein is the Archetypal Coagulase of the *Staphylococcus* Genus

Amy C. Pickering[1], Gonzalo Yebra[1], Bryan A. Wee[1,2], Alison MacFayden[1,3], Joana Alves[1], Gordon Gong[1], Mariya I. Goncheva[1,4], Lukas Muhlbauer[1,5], Robyn A. Cartwright[1], J. Ross Fitzgerald[1]
[1]Roslin Institute and Edinburgh Infectious Disease, University of Edinburgh, Edinburgh, United Kingdom. [2]The Usher Institute, University of Edinburgh, Edinburgh, United Kingdom. [3]Institute of Biodiversity Animal Health & Comparative Medicine, University of Glasgow, Glasgow, United Kingdom. [4]Department of Microbiology and Immunology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Canada. [5]Roger Land Building, University of Edinburgh, Edinburgh, United Kingdom

## Abstract

The bacterial genus *Staphylococcus* comprises a large group of pathogenic and non-pathogenic species associated with distinct host habitats. Traditionally, Staphylococci are differentiated into coagulase-positive (CoPS) and coagulase-negative Staphylococci (CoNS), based on the capacity to promote coagulation of plasma, a phenotype associated with the ability to cause disease. However, the genetic basis of this key diagnostic and pathogenic trait has not yet been established at genus level. Here, we examine the capacity of 59 representative staphylococcal species and subspecies to mediate coagulation of plasma from six host-species. Of the staphylococcal species tested, 12 mediated coagulation of plasma from at least one host species including one previously categorised as coagulase-negative (*Staphylococcus condimenti*). Comparative genomic analysis revealed that the coagulase phenotype correlated with the presence of a gene encoding the von Willebrand binding protein (vWbp) whereas only the *Staphylococcus aureus* complex encodes Staphylocoagulase (Coa), the widely-accepted mediator of Staphylococcal coagulation, and recombinant vWbp was sufficient to mediate host-specific coagulation of plasma. Importantly, *S. aureus* retains vWbp-dependent coagulase activity in a *S. aureus* strain deleted for *coa* whereas deletion of *vwb* in the coagulase-positive species *Staphylococcus pseudintermedius* results in complete loss of coagulase activity. Whole genome sequence-based phylogenetic reconstruction of the *Staphylococcus* genus revealed that the *vwb* gene has been acquired on at least four occasions during the evolution of the *Staphylococcus* genus and recombination has contributed to allelic variation that confers host-specificity. Taken together, we have determined the genetic and evolutionary basis of staphylococcal coagulation revealing vWbp to be its archetypal determinant.

## P343

# Gene relationships in an *Escherichia coli* pangenome

Rebecca Hall[1], Fiona Whelan[2], Elizabeth Cummins[1], Christopher Connor[1], Alan McNally[1], James McInerney[2]
[1]University of Birmingham, Birmingham, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom

## Abstract

The pangenome contains all genes encoded by a species, with the core genome present in all strains and the accessory genome in only a subset. Coincident gene relationships are expected within the accessory genome; the significant presence or absence of *geneA* given the presence or absence of *geneB*. Here, we analysed the accessory genome of an *Escherichia coli* pangenome consisting of 400 genomes from 20 sequence types to identify genes that display significant co-occurrence or avoidance patterns with one another. We present a complex network of genes that are either found together or that avoid one another more often than would be expected by chance, and show that these relationships vary by lineage. We highlight key influences behind these relationships, furthering our understanding of the dynamic nature of prokaryote pangenomes, and exemplify an application in antimicrobial resistance research.

## P346

# Mining the Escherichia coli pangenome for lineage specific metabolism

Elizabeth Cummins[1,2], Rebecca Hall[1], James McInerney[2], Alan McNally[1]
[1]University of Birmingham, Birmingham, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom

## Abstract

*Escherichia coli* is a diverse species spanning both common commensals of humans and animals and clinically important, major global pathogens. The metabolic capabilities of certain phenotypic groups can influence traits including colonisation, virulence and pathogenicity, but the extent to which these traits vary across lineages is unknown. To assess this, 20,577 genomes were used to construct pangenomes for 21 distinct E. coli sequence types. Core metabolic networks were subsequently built for each lineage. Using these reconstructions, we uncovered huge, lineage-specific differences in the core metabolic profiles. We have identified a set of metabolic reactions that are uniquely present in each lineage, and a set that are uniquely absent, highlighting key genes of interest in the search for adaptations that are lineage- and environment-specific. Our results suggest that variation in metabolic functions, deemed essential for the success of a lineage, will be key in future research to manipulate the abilities of specific *E. coli* lineages in their relevant environments.

**P347**

# Genomic analyses of *Bacteroides fragilis*: divisions one and two represent distinct species

Jamie English[1], Professor Sheila Patrick[2], Professor Garry Blakely[3], Professor Lesley Hoyles[4], Dr Linda Stewart[1]
[1]Institute for Global Food Security, School of Biological Sciences, Queen's University, Belfast, United Kingdom. [2]Wellcome-Wolfson Institute for Experimental Medicine, Queen's University, Belfast, United Kingdom. [3]School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom. [4]School of Science and Technology, Nottingham Trent University, Nottingham, United Kingdom

## Abstract

**BACKGROUND –** The Gram-negative anaerobe *Bacteroides fragilis* is an opportunistic pathogen localised to the human gastrointestinal tract. *B. fragilis* is divided into two groups – division I and II – that are distinguished by the presence/absence of the antimicrobial resistance genes *cepA* and *ccrA*, respectively.

**METHODOLOGY –** Publicly available genomes listed as *B. fragilis* (n=187) were subject to genomic characterization including average nucleotide identity (ANI), phylogenetic analysis, and resistome profiling. The faecal microbiota composition of a UK cohort (n=250) was also investigated to determine the prevalence of division I and II *B. fragilis*.

**RESULTS –** ANI and phylogenetic analyses indicated division I and II *B. fragilis* represent two distinct species; *B. fragilis sensu stricto* and *B. fragilis* A. Resistome screening confirmed the presence of antimicrobial resistance gene *adeF* among all *B. fragilis sensu stricto* genomes; it was absent from all *B. fragilis* A genomes. In one *B. fragilis sensu stricto* genome, we discovered a potentially novel *nim* gene likely to confer resistance to metronidazole. Initial faecal microbiota composition analyses of a UK cohort found *B. fragilis sensu stricto* (division I) and *B. fragilis* A represented at least 0.05% of the faecal microbiota in 211/250 and 56/250 individuals, respectively.

**CONCLUSION –** These findings reinforce the importance of analysing publicly available genome data to improve our knowledge of the phylogeny and prevalence of anaerobic bacteria and the potential for antimicrobial resistance. The identification of a potentially novel *nim* gene adds to the growing threat which the emergence of antimicrobial resistance poses to public health.

**P348**

# Utilisation of historical isolates to identify likely key genetic drivers in the long-term success of *Shigella* as pathogens

Rebecca Bennett[1], Dr Rebecca J Bengtsson[1], Dr Tim R Blower[2], Dr Malcolm J Horsburgh[1], Dr Kate S Baker[1]
[1]University of Liverpool, Liverpool, United Kingdom. [2]Durham University, Durham, United Kingdom

## Abstract

Bacteria of the genus *Shigella* are a major contributor to the global diarrhoea burden causing >60,000 deaths per annum globally. Increasing antibiotic resistance in *Shigella* and the lack of a licenced vaccine has led WHO to recognise *Shigella* as a priority organism for the development of new antibiotics. Understanding what drives the long-term persistence and success of this pathogen may identify targets for the global management of shigellosis with relevance to other enteric bacteria.

To identify key genetic drivers of *Shigella* evolution over the past 100 years, the unique and significant potential of historical bacterial genomes was utilised. The historical Murray collection, comprising several hundred pre-antibiotic era (1917 – 1954) *Enterobacteriaceae,* was used alongside more modern (1950s – 2018) isolates to initiate genome-wide association studies (GWAS). These studies aimed to identify genetic factors (SNPs and genes) significantly associated with time as a continuous variable. Key GWAS hits have putative activities that have intuitively beneficial roles for *Shigella* evolution, such as Rhs intracellular competition proteins and a putative resistance protein. Key hits then underwent variation and distribution analysis to examine the plausibility of their role in shaping *Shigella* populations and as potential targets for managing this pathogen.

Ongoing research aims are to compare GWAS hits among *Shigella* species and other *Enterobacteriaceae* to verify and identify further key genes across these pathogens. Thus, comprehensively identifying key genetic drivers of how AMR and other factors contribute to the long-term success of enteric bacteria, identifying potential targets for pathogen management.

**P349**

# Seeing the (random) forests for the genes: a machine learning approach to pangenome structure analysis

Maria Rosa Domingo-Sananes[1], James McInerney[2]
[1]Nottingham Trent University, Nottingham, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom

## Abstract

Factors such as phylogeny, adaptation and variable rates of gene gain and loss contribute to shaping the patterns of gene presence and absence in genomes. The result of these varying influences often manifests as extensive genomic variation between members of the same prokaryote species. In order to tease out the reasons why a gene might be present in a particular genome, we ask if we can predict whether or not a gene is likely to be present – based on the gene content of the rest of the genome. Ability to predict gene presence can imply the existence of interactions between genes, or shared selective pressures that act on those genes. To test this idea, we trained random forest classifiers to predict the presence of 2,592 variable genes with low phylogenetic signal (i.e. scattered across the phylogeny), in 409 genomes of the nitrogen-fixing bacterium *Rhizobium leguminosarum*. We obtained high-performing classifiers (accuracy and F-score >0.95) for around 10% of the genes. Using the training results from the classifiers we extract feature importances to construct weighted, directed networks which indicate how genes contribute to explain the presence of each other. We find groups of tightly connected genes that could signify biologically relevant relationships and propose experiments to test these predictions. We expect that this approach can be easily extended to include further predictive/explanatory variables, such as phenotypic and environmental information as well as core genome sequence variation.

## P350

# Different molecular routes to mat formation in environmental *Pseudomonas* isolates

Anuradha Mukherjee[1,2], Jenna Gallie[1]
[1]Max Planck Institute for Evolutionary Biology, Ploen, Germany. [2]Christian-Albrechts University, Kiel, Germany

## Abstract

Many bacterial species secrete polymers and form mat-like structures. These mats can be useful (*e.g.*, in bioremediation), or problematic (*e.g.*, in hospital settings). The molecular bases of mat formation have been investigated in a number of species, including various Pseudomonads. One well-characterized example is the plant symbiont *Pseudomonas fluorescens* SBW25. Laboratory populations of SBW25 readily acquire mutations in one of three regulatory loci (*wsp*, *aws*, *mws*), leading to the over-production of the secondary messenger cyclic-di-GMP. In turn, this activates the production of a cellulose-like polymer, the major structural component of the SBW25 mat. Here, we dissect and compare the molecular mechanisms of mat formation in two further plant-associated Pseudomonads: *P. simiae* PICF7 and *P. fluorescens* A506. We find that both PICF7 and A506 are capable of mat formation in the laboratory, by distinct molecular routes. Mat formation in PICF7 involves mutations in *wsp*, *aws,* or *mws* that serve to activate the production of Pel (as opposed to cellulose in SBW25). Contrastingly, A506 mat formation does not require mutation of *wsp*, *aws*, or *mws* (despite their retention in the genome). Instead, our results are consistent with a readily reversible, non-mutational route to polymer production and mat formation in A506. Overall, our results demonstrate the presence of multiple molecular routes to mat formation among environmental, plant-associated *Pseudomonas* strains.

## P351

# Using gene-gene co-occurrence and avoidance relationships to understand pangenomes via the publicly available Coinfinder software

Fiona Whelan[1], Arooj Qamar[2], Joseph McPhee[2], James McInerney[1]
[1]University of Nottingham, Nottingham, United Kingdom. [2]Ryerson University, Toronto, Canada

## Abstract

Pangenomes describe the totality of genes (both core and accessory) that are present in a set of evolutionarily related strains. In some species, the pangenome contains an excess of accessory genes and is said to be *open*. We hypothesize that open pangenomes are maintained by selection, and that, as such, gene gain and loss will not be random, but instead will reflect functional patterns within the pangenome. If this hypothesis is correct, we would expect to see gene associations (i.e. gene-gene co-occurrence) between functionally related genes within microbial pangenomes. To test this hypothesis, we examined 209 *Pseudomonas sp.* genomes, and, using new software called Coinfinder, identified gene-gene co-occurrence pairs in addition to the connect components (i.e. gene sets) that they subsequently form. We find examples of gene associations which reflect what is known biologically (for example, the co-occurrence of transporter genes), as well as gene associations between hypothetical proteins. We found that, when compared to random gene pairs, co-occurring gene pairs shared more functionality with each other, were more often expressed together, and were more likely to produce proteins that interact with each other. We did not find strong syntenic signatures of co-occurring gene pairs. Further, we show similar results in other microbial pangenomes, including those of *Streptococcus pneumoniae*, *Halobacterium sp.*, and *Shigella sp*. Together, these findings show that pangenomes contain functionally related genes that co-occur with each other and suggests that, overall, microbial pangenomes are structured under the constraints of natural selection.

## P352

# Whole genome sequencing and comparative analysis of a novel *Chlamydia psittaci* strain

Morag Livingstone[1], Paolo Ribeca[2], Delphine Beeckman[3], Arie van der Ende[4], Yvonne Pannekoek[4], Daisy Vanrompay[3], David Longbottom[1]
[1]Moredun Research Institute, Edinburgh, United Kingdom. [2]Biomathematics & Statistics Scotland, Edinburgh, United Kingdom. [3]University of Ghent, Ghent, Belgium. [4]University of Amsterdam, Amsterdam, Netherlands

## Abstract

Background

*Chlamydia abortus* and *Chlamydia psittaci* are important pathogens of livestock and avian species, respectively. While *C. abortus* is recognized as descended from *C. psittaci* species, there is emerging evidence of strains that are intermediary, including *C. psittaci* strain 84/2334 that was isolated from a parrot.

Methods

Strain 84/2334 was analysed by multi-locus sequence typing of seven housekeeping genes and by analysis of five species-discriminant proteins. The strain was *de novo* sequenced for comparison with representative strains from chlamydial species. 16S rRNA and whole genome phylogenetic analyses, network and recombination analyses, and analyses of key virulence-associated genes were conducted to explore its evolutionary relationship with both *C. psittaci* and *C. abortus*.

Results

Typing, 16S rRNA and whole genome phylogenetic analyses, as well as network and recombination analyses showed that strain 84/2334 clusters closely with *C. abortus*. Analyses also suggested a closer evolutionary relationship with classical *C. abortus* strains, than to two other avian *C. abortus* or *C. psittaci* strains. Analyses of virulence-associated genes that exhibit greatest diversity within chlamydial species, reveal greater diversity than present in sequenced *C. abortus* genomes. The strain also possesses an extrachromosomal plasmid, as found in *C. psittaci* species but absent from all sequenced classical *C. abortus* strains.

Conclusion

The results are consistent with 84/2334 being a *C. abortus* ancestral strain, suggesting that it should be reclassified as *C. abortus*. The identification of a *C. abortus* strain bearing an extra-chromosomal plasmid has implications for plasmid-based transformation studies providing a potential route for future vaccine development.

## P354

# Environment and the Evolutionary Trajectory of Horizontal Gene Transfer.

Rama Bhatia[1], Hande Kirit[1,2], Jonathan Bollback[1]
[1]University of Liverpool, Liverpool, United Kingdom. [2]University of Oklahoma, Oklahoma, USA

## Abstract

Gene acquisition through horizontal gene transfer (HGT) may either exert a beneficial, neutral or deleterious fitness effect on the recipient cell thereby determining the evolutionary fate of the newly transferred gene. The distribution of fitness effects (DFE) thus is a fundamental predictor of the outcome of an HGT event.

The environment plays a considerable role in altering the fitness of a horizontally transferred gene. We have studied the DFE of genes transferred from *Salmonella enterica* serovar Typhimurium to *Escherichia coli* in six environments, that potentially represent the conditions experienced by the two species. The data suggests high variability, with some genes becoming deleterious in one environment, while becoming neutral or even beneficial in another, suggesting that fluctuating environments may increase the likelihood of HGT.

The present study focuses on the DFE of genes transferred from *Escherichia coli* to macrophage-associated *Salmonella enterica* serovar Typhimurium ST4/74 in five environments, that mimic conditions within macrophages. In addition to the external environment, we are also looking at, how changes in the intrinsic environment of a cell, after an HGT event, could affect fitness. Functional similarity of the horizontally transferred gene to the endogenous copy can cause an imbalance due to increased protein dosage, thereby leading to a negative fitness effect. By comparing the growth rates of each ortholog gene with the wild type, in the five environments, we can elucidate when gene dosage acts as a barrier to HGT, helping us to understand the relationship of environmental quality and HGT, which is of evolutionary importance.

## P355

# Making virus taxonomy accessible

Donald Smith[1,2], Richard Orton[3], Murilo Zerbini[4], Elliot Lefkowitz[5], Sead Sabanadzovic[6], Peter Simmonds[1], Stuart Siddell[7]
[1]University of Oxford, Oxford, United Kingdom. [2]University of Edinburgh, Edinburgh, United Kingdom. [3]University of Glasgow, Glasgow, United Kingdom. [4]Universidade Federal de Viçosa, Vicosa, Brazil. [5]University of Alabama at Birmingham, Birmingham, USA. [6]Mississippi State University, Starkville, USA. [7]University of Bristol, Bristol, United Kingdom

## Abstract

The International Committee on Taxonomy of Viruses (ICTV) is responsible for approving changes to the taxonomy of viruses. These changes arise from proposals made to the ICTV, usually by one of its 101 Study Groups, each of which is responsible for a particular virus family or families. The result of this international, voluntary effort is a searchable and structured list of current virus taxonomy that can be accessed on the ICTV website (www.ictv.global). The website also makes available the virus metadata resource (VMR), a spreadsheet with exemplar isolates and GenBank accession numbers for members of each species, as well as the ICTV Report which provides more detail about the taxonomy, structure and biology of each virus family. The online publication mode allows taxonomic changes and relevant research findings to be quickly reflected in the Report, and also means that the sequence alignments and phylogenetic trees, upon which taxonomic decisions often depend, are available as resources for all to use. A two-page summary of each Report chapter is published as an Open Access article by the Journal of General Virology as a Virus Taxonomy Profile. It remains a challenge to make these freely available resources comprehensive because of the pace of taxonomic change; over the last decade the number of virus families has almost doubled from 87 to 168. In meeting this challenge the ICTV will continue to rely on the enthusiasm, expertise and efforts of virologists from around the world.

## P356

# The use of comparative genome analysis for the development of subspecies-specific PCR assays for *Mycobacteroides* (*Mycobacterium*) *abscessus*

Winifred Akwani[1], Arnoud van Vliet[2], Jordan Joel[2], Mark Chambers[1,2], Suzie Hingley-Wilson[1]
[1]University of Surrey, School of Biosciences and Medicine, Guildford, United Kingdom. [2]University of Surrey, School of Veterinary Medicine, Guildford, United Kingdom

## Abstract

The *Mycobacteroides abscessus* complex (MABC) (comprising of the subspecies *abscessus*, *bolletii* and *massiliense*) has recently emerged as an important threat to cystic fibrosis patients. Accurate identification of MABC at the subspecies level is vital for correct antibiotic therapy. We have used comparative genome analysis to develop a sensitive and specific PCR-based test to rapidly differentiate between the *M. abscessus* subspecies. A total of 1,531 *M. abscessus* complex genomes were downloaded from Genbank and assigned to subspecies based on the core genome SNPs and feature frequency profiling. Pangenome analysis of 318 Prokka-annotated MABC genomes using Roary and Scoary enabled identification of genes specific for each MABC subspecies. These genes were further used for the design of PCR primers to be used diagnostically. Candidate primer pairs were tested *in silico* against the 1,212 genomes that were not used for comparative genomics. *In silico* PCRs based on the best primer pairs generated a product for 100%, 100% and 96.4% of the MABC subspecies *abscessus*, *bolletii* and *massiliense*, respectively, while none showed false-positive reactions with the other two subspecies. These PCR primers were subsequently tested experimentally and worked as predicted using genomic DNA derived from MABC species and related *Mycobacteroides* species and could be used in a multiplex format. In conclusion, we have developed a rapid, sensitive, multiplex PCR-assay for discriminating MABC subspecies that could aid in understanding the epidemiology of *M. abscessus*, diagnosis and therapeutic regimens.

## P358

# Using transcriptomics and Hi-C sequencing to explore the impact of large-scale genome rearrangement in *Salmonella*

Emma Ainsworth[1], Lisa Crossman[2], Liam Tucker[1], Marie-Anne Chattaway[3], Satheesh Nair[3], Benjamin Evans[2], Gemma Langridge[1]
[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom. [3]Public Health England, London, United Kingdom

## Abstract

Background: By routinely using long read sequencing, we have observed that *Salmonella enterica* genomes tolerate changes in the gross-scale ordering and orientation of genome fragments around the seven ribosomal operons. Here, we investigated the impact of rearrangement in a laboratory strain and in clinical isolates of *S. enterica* serovar Typhi.

Methods: We confirmed the presence of different genome arrangements using long read sequencing (Oxford Nanopore Technologies) and *socru* software, for typing structural variants in complete bacterial genomes. The impact of rearrangement was investigated using transcriptomics for differential gene expression and proximity-ligation (Hi-C) sequencing to assess the effect on 3D chromosome folding.

Results: We identified several rearrangements in the laboratory strain, which were induced by long-term culture. We also identified novel arrangements in a collection of clinical isolates from Public Health England. By comparing the same strain in different arrangements, we observed reduced gene expression in genome fragments with increased distance from the origin of replication. Mapping of chromosome contacts from our Hi-C data also demonstrated a clear change in chromosome folding between different arrangements.

Conclusion: Gross-scale rearrangement of the order and orientation of genome fragments impacts the physical folding of the chromosome and expression of genes on the rearranged fragments. This may therefore represent a mechanism by which bacteria can adapt to environmental pressures.

## P360

# genomeRxiv: a microbial whole-genome database and diagnostic marker design resource for classification, identification, and data sharing

Leighton Pritchard[1], Parul Sharma[2], Reza Mazloom[2], Tessa Pierce[3], Luiz Irber[3], Bailey Harrington[1], Lenwood Heath[2], C Titus Brown[3], Boris Vinatzer[2]
[1]University of Strathclyde, Glasgow, United Kingdom. [2]Virginia Tech, Blacksburg, USA. [3]UC Davis, Davis, USA

## Abstract

genomeRxiv is a newly-funded US-UK collaboration to provide a public, web-accessible database of public genome sequences, accurately catalogued and classified by whole-genome similarity independent of their taxonomic affiliation. Our goal is to supply the basic and applied research community with rapid, precise and accurate identification of unknown isolates based on genome sequence alone, and with molecular tools for environmental analysis.

The DNA sequencing revolution enabled the use of cultured and uncultured microorganism genomes for fast and precise identification. However, precise identification is impossible without

1. reference databases that precisely circumscribe classes of microorganisms, and label these with their uniquely-shared characteristics
2. fast algorithms that can handle the volumes of genome data

Our approach integrates the highly-resolved classification framework of Life Identification Numbers (LINs) with the speed and computational efficiency of sourmash and k-mer hashing algorithms, and the precision and filtering of average nucleotide identity (ANI). We aim to construct a single genome-based indexing scheme that extends from phylum to strain, enabling the unique and consistent placement of any sequenced prokaryote genome.

genomeRxiv includes protocols for confidentiality, allowing groups to identify and announce the identities of newly-sequenced organisms without sharing genome data directly. This protects communities working with commercially- and ethically-sensitive organisms (e.g. production engineering strains, potential bioweapons, and to enable benefit sharing with indigenous communities).

genomeRxiv will also provide online capability to design molecular diagnostic tools for metabarcoding and qPCR, to enable tracking of specific groupings of bacteria directly in the environment.

## P361

# Comprehensive Evaluation of CAZyme Prediction Tools in Fungal and Bacterial Species

Emma Hobbs[1], Dr Tracey Gloster[1], Dr Sean Chapman[2], Dr Leighton Pritchard[3]
[1]University of St Andrews, St Andrews, United Kingdom. [2]The James Hutton Institute, Dundee, United Kingdom. [3]University of Strathclyde, Glasgow, United Kingdom

### Abstract

Carbohydrate Active enZymes (CAZymes) are required for pathogen recognition, signalling, structure and energy metabolism. Annotation of a microorganism's CAZyome (all CAZymes in the proteome) provides functional information that aids identifying novel and useful enzymes relevant, for instance, to biofuel production.

CAZy is the most comprehensive CAZyme database [Lombard et al., 2013], but does not provide methods for automated annotation retrieval or submitting sequences for annotation. The CAZyme prediction tools dbCAN [Zhang et al., 2018], CUPP [Barrett and Lange, 2019] and eCAMI [Xu et al., 2019] annotate CAZymes. However, these do not integrate directly with CAZy, and have not been evaluated on a common high-quality benchmarking dataset.

Here, I report the absolute and relative CAZyme prediction accuracy for dbCAN, eCAMI and CUPP, assessed against the same high-quality benchmark sequence set of fungal and bacterial proteins.

To evaluate the tools and identify the comprehensive CAZyome in candidate species, I developed the bioinformatic pipeline 'pyrewton' (DOI:10.5281/zenodo.3876218). To generate a high-quality benchmarking dataset for the evaluation, I retrieved 200 randomly selected CAZymes, and 200 non-CAZyme negative controls with the highest sequence similarity to CAZymes, from each candidate genome. As CAZymes may belong to multiple families, both binary and multi-label classifications were assessed, which has not been addressed in previous evaluations.

Ground truths for the evaluation were retrieved using 'cazy_webscraper' (DOI:10.5281/zenodo.4300858), which automates customisable scraping of CAZy, producing a local SQLite database. Cazy_webscraper can populate the database with protein sequences from GenBank, and structure files from RCSB.

## P362

# cazy_webscraper: for creating a local CAZy database

Emma Hobbs[1], Dr Leighton Pritchard[2], Dr Sean Chapman[3], Dr Tracey Gloster[1]
[1]University of St Andrews, St Andrews, United Kingdom. [2]University of Strathclyde, Glasgow, United Kingdom. [3]The James Hutton Institute, Dundee, United Kingdom

### Abstract

Carbohydrate Active enZymes (CAZymes) are pivotal in pathogen recognition, signalling, structure and energy metabolism. CAZy (www.cazy.org) is the most comprehensive CAZyme database [Lombard et al., 2013], cataloguing CAZymes into sequence-based families. However, CAZy does not provide methods to automate annotation retrieval, instead requiring users to manually navigate through numerous web pages.

Cazy_webscraper is a command-line tool that retrieves data from CAZy for local storage in an SQLite database, enabling interrogation of the downloaded dataset with simple or complex queries using SQL. Therefore, users can thoroughly explore CAZy data in a manner unachievable through the CAZy website. For example, querying the database to identify CAZymes within specific families, derived from selected species, and with structural records in RCSB for inclusion in dynamic modeling to identify key catalytic residues.

Unlike previous scrapers, cazy_webscraper can be configured to scrape the entire database, or only user-specified CAZy classes and/or families. This reduces demand on the CAZy server and user waiting times. Scraping GH10, containing 4938 CAZymes, takes 4 minutes and 54 seconds using AMD FX-6300 (3.5GHz) processor, 16GB RAM and an SSD drive. A taxonomy filter can limit the scrape to specific genera, species and/or strains.

Cazy_webscraper can populate the local database with protein sequences from GenBank, and download protein structures from RCSB, enabling sequence- and structure-based analyses.

Cazy_webscraper creates a timestamped dataset, encouraging reproducibility, and automates integrating annotation, sequence and structural data, improving ease of use. This facilitates complex bioinformatic analyses of large CAZyme datasets, including phylogenetic analysis, and identification of novel CAZymes.

## P363

# Molecular characteristics of hypervirulent *Klebsiella pneumoniae* induced ventilator associated pneumonia in Assiut University Hospital, Egypt

Hadeel Elzeny[1], Wegdan Mohamad[2], Enas Daef[2], Omnia El-Badawy[2], Lamiaa Shaaban[3], Sherine Aly[2]
[1]Assiut International Center of Nanomedicine, Assiut, Egypt. [2]Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt. [3]Chest Department, Assiut University Hospital, Assiut, Egypt

## Abstract

Hypervirulent *Klebsiella pneumoniae* (hvKP) is a newly discovered strain that shows unique characteristic compared to the classical *K. pneumoniae* (cKP). The new strain is a way more virulent than its predecessor with its ability to cause severe infection and metastasis in non-immunocompromised patients. Many features of hvKP are still unclear and need more investigations especially in Upper Egypt, where no previous studies were performed.

The current work is a retrospective study included 59 specimens collected from patients diagnosed with ventilator associated pneumonia (VAP) caused by *K. pneumoniae*. Antibiotic susceptibility profile was determined and capsular serotype genes K1 and K2, as well as virulence genes (*c-rmpA*, *p-rmpA*, *iucA*, *kfu*, *iroB* and *iroN)* were detected. Clonal relatedness was determined by pulsed field gel electrophoresis (PFGE).

The results showed that hvKP accounted for nearly two–thirds of *K. pneumoniae* induced VAP. The prevalence of *p-rmpA, iucA* and *kfu* genes in hvKP strains were significantly higher, while the prevalence of genotype K2 showed no significance difference between the two types. All hvKP and cKP strains were MDR with no statistical difference in the resistance pattern. PFGE pattern revealed multi clonal origin for most of the isolates.

In conclusion, hvKP predominate as the causative agent of VAP in Assiut University Hospital. These hvKP strains were thought to develop from evolution of cKP through acquisition of virulence plasmid. Common PFGE cluster and antibiotic susceptibility profile between some cKP and hvKP solidify the theory of evolution.

***Keywords:*** cKP, hvKP, Upper Egypt, VAP

## P364

# Host Prediction of *S. typhimurium* sequences via machine learning

Antonia Chalka[1], Tim Dallman[2], David Gally[1], Mark Stevens[1]
[1]University of Edinburgh, Edinburgh, United Kingdom. [2]Public Health England, London, United Kingdom

## Abstract

*Salmonella enterica* is a diverse pathogen with over 2600 serovars that differ in host-specificity. Generalist serovars like *S. typhimurium* infect a wide range of hosts, while host-specific serovars have a narrower range. The genetic factors underlying host specificity are complex, but the availability of thousands of sequences and advances in machine learning have made it possible to build host prediction models which would be invaluable in outbreak control.

Previous studies have already showcased the potential of using machine learning to examine S. enterica host specificity, so we focused on building a host-specificity prediction model based on a wide range of genomic features. We collected 3300 *S. Typhimurium* assembled sequences isolated from humans, swine, bovine and poultry from the USA. SNPs, protein variants, AMR profiles and intergenic regions were extracted from high-quality assemblies, filtered, and fed into RandomForest machine learning models.

The models based on protein variants and intergenic sequences had a similar average accuracy, comparable with previous studies (80-90%). AMR profile models had the lowest predictive accuracy (60-80%) with great variation within hosts. Poultry predictions had the highest accuracy (>90%), whereas bovine and human predictions had the lowest, in line with our dataset's phylogeny, which includes a large distinct poultry clade, and 'mixed' clades of human and bovine.

Future plans aim to refine our model building approach by extracting and combining the most important features across the different genetic categories, as well as expanding and re-applying the methodology above to other serovars, such as *S. Enteritidis*.

## P365

# Epigenetic Modification Impacts on Gene Regulation in *Streptococcus pneumoniae*

Yasmeen Althari, Yongkui Chen, Richard Haigh, Marco Oggioni
university of leicester, leicester, United Kingdom

## Abstract

Methylation by the type I restriction modification system (RMS) SpnIII in *Streptococcus pneumoniae* is hypothesised to regulate gene expression via epigenetic changes (Manos). The phase variable SpnIII generates six host specificity determinants (*hsdS*) alleles through site-specific recombination (DeSteCroix), each allele correspond with different methylation pattern.

In addition to known functions of the RMS in restricting phage infection (Furi) and transformation (Kwun), we have now tested impact on gene expression. RNAseq was used to analyse *S. pneumoniae* strains expressing a single *spnIII* allele (*spnIII*A, *spnIII*B or *spnIII*E) to determine differences in gene expression profiles. The data have identified six genes which show differential expression and have a methylation site mapping to their predicted promoter region. Three synthetic promoters with the wild type and altered methylation target site were cloned in front of a luciferase gene in strains expressing a single *spnIII* alleles. For the first three sets of constructs analysed, data indicate that the methylated promoters show a three- to twenty-fold higher activity compared to non-methylated promoters. Results obtained were further confirmed by qPCR analysis. Preliminary data using drugs targeting DNA topoisomerases indicate that the mechanism by which methylation impact gene expression could be related to DNA topology. These data demonstrate for the first-time RMS dependent variations in gene expression and propose an alternative mechanism for this epigenetic regulation.

References:

- Manso, Nature Communication, 2014; 5:5055.
- De Ste Croix, J. Bacteriol. 2019; 201(15). pii: e00233-19.
- Furi, J. Bacteriol. 2019; 201(19). pii: e00370-19.
- Kwun, Nucleic Acids Res. 2018; 46(21):11438-11453.

## P366

# The genetics of prey susceptibility to myxobacterial predation.

Natashia Sydney, David Whitworth
Aberystwyth University, Aberystwyth, United Kingdom

## Abstract

Bacterial predation is a ubiquitous and fundamental biological process, which influences microbial ecosystems. Among the best characterised bacterial predators are the myxobacteria, which include the model organism *Myxococcus xanthus*. Predation by *M. xanthus* involves the secretion of antibiotic metabolites and hydrolytic enzymes, which results in the lysis of prey organisms and release of prey nutrients into the extracellular milieu. Due to the generalist nature of this predatory mechanism, *M. xanthus* has a broad prey range. Potential prey organisms have evolved a range of behaviours which protect themselves from attack by predators. In recent years, several investigations have studied the molecular responses of a broad variety of prey organisms to *M. xanthus* predation. It seems that the diverse mechanisms employed by prey belong to a much smaller number of general 'predation-resistance' strategies. As previous molecular studies of prey susceptibility have focused on individual genes/metabolites, we have also undertaken a genome-wide screen for genes of *Pseudomonas aeruginosa* which contribute to its ability to resist predation. *P. aeruginosa* is a World Health Organisation priority 1 antibiotic resistant pathogen. Using a library of nearly 5,500 defined transposon insertion mutants we screened for 'prey genes', which when mutated allowed increased predation by a fluorescent strain of *M. xanthus*. A set of candidate 'prey proteins' were identified, which shared common functional roles and whose nature suggested that predation-resistance by *P. aeruginosa* requires an effective metal/oxidative stress system, an intact motility system, and mechanisms for de-toxifying antimicrobial peptides.

**P367**

# Functional genomic approaches to manipulating *Bordetella pertussis* outer membrane synthesis for vaccine development.

David McCulloch
University of Bath, Bath, United Kingdom. GSK vaccines, Siena, Italy

## Abstract

Vaccines against *Bordetella pertussis* have been available since the 1940s, however, disease resurgence in highly vaccinated populations demonstrates current vaccine strategies are failing to prevent disease transmission. Therefore, there is a need for novel vaccine antigen development to improve current vaccines for controlling pertussis infection rates. *B. pertussis* outer membrane vesicles (OMV) have potential to be utilised as a novel vaccine component. However, for OMVs to be produced at a level required for use in vaccine development, the mechanisms of OMV biogenesis need to be elucidated.

We have utilised Transposon directed insertion sequencing techniques (TraDIS) to identify non-essential genes involved in outer membrane synthesis and stability as targets for mutation to increase OMV synthesis. Through mutagenesis of these targets, we have investigated the effect of gene knockout on bacterial morphology, viability and vesiculation.

TraDIS analysis of *B. pertussis* displayed the *mre/mrd* operon, involved in peptidoglycan synthesis, as only conditionally essential during plate growth, dependent upon the activation of the BvgAS two component system, but entirely non-essential during bacterial growth in liquid culture. *Mre/mrd* knockout was found to produce coccus-shaped bacteria with viability comparable to wildtype *B. pertussis.* Using SEM, knockout of *mrdA, mreB* and *mreC* was revealed to result in accumulation of extracellular components.

Overall, these findings reveal that the *mre/mrd* operon can be genetically manipulated in *B. pertussis*, altering outer membrane synthesis without reducing bacterial viability. Future analysis into strain vesiculation will elucidate whether *mre/mrd* mutagenesis could be utilised to produce OMVs for vaccine development.

## P368

# Dissecting meningococcal disease and carriage traits using high throughput phenotypic testing

Robeena Farzand[1], Evangelos Mourkas[2], Megan De Ste Croix[1], Neelam Dave[1], Abdullahi Jama[1], Neil Oldfield[3], Jay Lucidarme[4], David Turner[3], Ray Borrow[4], Luisa Martinez-Pomares[3], Samuel Sheppard[2], Christopher Bayliss[1]
[1]University of Leicester, Leicester, United Kingdom. [2]University of Bath, Bath, United Kingdom. [3]University of Nottingham, Nottingham, United Kingdom. [4]Meningococcal Reference Unit, Public Health England, Manchester, United Kingdom

## Abstract

Despite on-going vaccination programmes, *Neisseria meningitidis* is a major cause of septicaemia and meningitis. In 2017-18, the MenW and MenY capsular groups caused 38% of all UK invasive meningococcal disease cases. Current policy is to generate genome sequences of all meningococcal disease isolates. Using this resource, we aim to determine how genetic variation contributes to phenotypic differences between carriage and disease isolates. We have adapted assays, mimicking carriage and disease behaviours, for high-throughput phenotypic testing of 335 MenW cc11 and MenY cc23 isolates. We are currently testing MenW cc11 disease and carriage isolates for cytotoxicity in a human lung epithelial cell line, growth in media and biofilm formation. Phenotypic differences are utilised as inputs for Genome Wide Association Studies enabling linkage of specific genomic variants, or variant combinations, with phenotypic variation. Genomic data include whole genome sequences and repeat-mediated phase variation states.

The MenW cc11 isolates span two known phylogenetic clusters, original and 2013. Our preliminary data from high-throughput growth and biofilm assays showed no significant differences between these groups or sources (disease versus carriage); however, variations were observed within groups with, for example, distinctive cytotoxicity or biofilm differences between isolates. These variations may reflect physiological divergence due to minor genetic modifications between highly phylogenetically-related strains. Our assay systems are robust, reproducible, and easily scalable for efficient high-throughput genotypic and phenotypic testing. Thus, large-scale screening of phenotypic variation for infectious diseases is achievable and harnessable for cost-effective, direct evolutionary and epidemiological studies.

## P369

# The caecal and faecal dropping microbiota of pet hens

Natalie Barratt, Julian Ketley
University of Leicester, Leicester, United Kingdom

### Abstract

The hen gut microbiota (GM) varies in its composition and function depending on the location in the gut. The caecal microbiota (CM) is primarily responsible for the extensive fermentation and digestion of complex carbohydrates. Compared to the faecal microbiota (FM), the CM is often more diverse, rich and stable. The population structure and the functionality of the GM has been linked to many aspects of health, and correlations between the GM and the litter microbiota (LM) have also been noted. The microbiota of hens raised in small flocks in people's private gardens (PG) or allotment plots (AP) remains largely unresearched, this project aims to help rectify this.

This study uses 16S rDNA V4 sequencing to characterise the FM, CM and LM of seven PG flocks and seven AP flocks located in the east midlands. Flock health and management practises were noted. From each flock, three caecal droppings, three faecal droppings and a litter sample were analysed. The MiSeq SOP from Mothur was adapted to perform the amplicon-based microbiome analysis. The FM, CM and LM are distinct from each other in their core phyla relative abundances, on average the CM were richer. The FM is dominated by the phyla Firmicutes (45%), Proteobacteria (17%) and Bacteroidetes (15%) in order of abundance, whereas the CM is dominated by Bacteroidetes (40%), Firmicutes (28%) then Proteobacteria (13%), and the LM by Proteobacteria (36%), Firmicutes (25%) then Bacteroidetes (24%). There is no significant difference between the microbiotas of hens from PG and AP flocks.

## P370

# Transcriptional profiles of *Streptococcus pneumoniae* associated with adaptation to the nasopharynx environment.

Thomas E Barton, Angharad E Green, Daniel R Neill
Department of Clinical Infection Microbiology & Immunology, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom

## Abstract

*Streptococcus pneumoniae* is a major cause of global morbidity and mortality. It behaves as a commensal in the host nasopharynx, but can become pathogenic, invading the lungs, blood, and meninges. As such, identification of pneumococcal virulence and colonisation factors remains a major research objective. We previously described an experimental evolution approach for the identification of pneumococcal genes that make niche-specific contributions to fitness and virulence. Sequential passage of pneumococci through mouse models of nasopharyngeal-carriage and pneumonia was performed, generating bacterial lineages adapted to the nasopharynx and lungs, respectively.

Using RNA-Seq differential gene expression analysis, this study compared the transcriptomic profile of a nasopharynx-evolved pneumococcal lineage that showed evidence of enhanced nasopharyngeal colonisation potential, with a lab-adapted ancestor strain. Here, we describe how the genomic adaptations acquired by this lineage, and which we have demonstrated facilitate survival in the nasopharynx, can influence bacterial gene expression.

One key finding was the identification of five adjacent upregulated genes, representing a putative pneumococcal operon. These poorly characterised genes are predicted to encode a carbohydrate-scavenging pathway. Expression of the operon within the nasopharynx may facilitate sugar acquisition from host glycoproteins. Of note, the nasopharynx evolved pneumococcal lineage carries a single nucleotide insertion mutation immediately adjacent to the -10 element of the operon predicted promotor sequence. This may contribute to increased operon gene expression in the nasopharynx-adapted pneumococcus, thereby enhancing colonisation potential. Confirmatory mechanistic investigations are underway, which will aid the identification of pneumococcal virulence factors involved in the commensal to pathogen switch.

**P371**

# Understanding the nature of *Vibrio cholerae* circulating prior to and during the mass vaccination campaign in the displaced Rohingya Muslim population in Bangladesh

Alyce Taylor-Brown[1], Mokibul Hassan Afrad[2], Ashafrul Islam Khan[2], Md. Taufiqual Islam[2,3], Nabid Anjum Tanvir[2], Nicholas R. Thomson[1,4], Firdausi Qadri[2]
[1]Wellcome Sanger Institute, Hinxton, Cambridge, United Kingdom. [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh. [3]Griffith University, Gold Coast, Australia. [4]London School of Hygiene and Tropical Medicine, London, United Kingdom

## Abstract

Following the ongoing forced displacement of Myanmar nationals into Bangladesh, the population of Cox's Bazar has increased drastically. Due to the already high population density coupled with poor living conditions, these Rohingya refugees (Forcibly Displaced Myanmar nationals; FDMNs) and the host population of Bangladeshi Nationals (BGDN) were at increased risk of disease including cholera. In 2014, sentinel surveillance of diarrhoea patients revealed that cholera, caused by the Gram-negative bacterium *V. cholerae*, was prevalent in the region, but confined to localised sporadic outbreaks. In response, a mass vaccination campaign was undertaken in 2017-2018 to control case numbers and prevent a potential epidemic in this at-risk population.

In this study, we sought to understand the dynamics of cholera in Cox's Bazar, and to determine the epidemic risk of the strains circulating in this region. We sequenced 222 *V. cholerae* isolates obtained from stool collected from ten surveillance sites throughout Cox's Bazar and the refugee camps (RCs) between 2014 and 2019, spanning the pre- and post-vaccination periods. 19 FDMNs and one BGDN residing in the RCs were vaccinated.

Phylogenetic analysis of these genomes in the context of published genomes showed that these isolates clustered within the high risk "7PET" lineage – responsible for the current cholera pandemic – branching alongside *V. cholerae* isolated in nearby Dhaka and Kolkata between 2015 and 2018.

Our data show that the mass vaccination campaign was successful in preventing a population expansion of the pandemic clone, and hence stemmed epidemic spread of *V. cholerae* in Cox's Bazar.

**P374**

# Landscape genomics of *Escherichia coli* in livestock-keeping households across a rapidly developing urban city

Dishon Muloi[1,2], Bryan Wee[1], Deirdre McClean[1], Melissa Ward[1,3], Louise Pankhurst[3], Hang Phan[3], Alasdair Ivens[1], Velma Kivali[2], Alice Kiyonga[2], Christine Ndinda[2], Nduhiu Gitahi[4], Tom Ouko[5], James Hassell[2,6], Titus Imboma[7], James Akoko[2], Maurice Karani[2], Samuel Njoroge[5], Patrick Muinde[2], Yukiko Nakamura[8], Lorren Alumasa[2], Erin Furmaga[9], Titus Kaitho[10], Elin Öhgren[11], Fredrick Amanya[2], Allan Ogendo[2], Daniel Wilson[3], Judy Bettridge[12], John Kiiru[5], Catherine Kyobutungi[13], Cecila Tacoli[14], Erastus Kang'ethe[4], Julio Davila[15], Sam Kariuki[5], Timothy Robinson[16], Jonathan Rushton[6], Mark Woolhouse[1], Eric Fèvre[2,6]
[1]University of Edinburgh, Edinburgh, United Kingdom. [2]International Livestock Research Institute, Nairobi, Kenya. [3]University of Oxford, Oxford, United Kingdom. [4]University of Nairobi, Nairobi, Kenya. [5]Kenya Medical Research Institute, Nairobi, Kenya. [6]University of Liverpool, Neston, United Kingdom. [7]National Museums of Kenya, Nairobi, Kenya. [8]Hokkaido University, Sapporo, Japan. [9]Columbia University, New York, USA. [10]Kenya Wildlife Service, Nairobi, Kenya. [11]Uppsala University, Uppsala, Sweden. [12]University of Greenwich, Greenwich, United Kingdom. [13]African Population Health Research Centre, Nairobi, Kenya. [14]International Institute for Environment and Development, London, United Kingdom. [15]University College London, London, United Kingdom. [16]Food and Agriculture Organization of the United Nations, Rome, Italy

## Abstract

The practice of livestock keeping in urban settlements found in lower to middle income countries has been suggested as a contributing factor to the spread of antimicrobial resistance between humans and animals. In one the largest genomic epidemiological studies of this nature to date, we sampled the ubiquitous commensal bacteria *Escherichia coli* from humans, livestock, and wildlife from 99 households across Nairobi, Kenya to get a deeper understanding of the distribution of bacteria among hosts. Using whole genome sequencing, we analysed the population structure of 1,338 *E. coli* isolates collected from these different hosts. Our findings show that strain sharing predominantly occurs within the household, shaped by host type. We find that strain sharing between humans and livestock does occur, but only very rarely and is linked to animal husbandry. We also find that sharing of AMR genes does not correlate directly with sharing of bacterial isolates with the majority of shared isolates identified as pan-susceptible. Resistome similarity, however, was more prominent in certain types of livestock, such as poultry. This, in our knowledge, is the most comprehensive study employing a structured sampling approach across human, livestock and wildlife compartments to understand the population dynamics of *E. coli* and their resistance genes in a single city. Our results have significance for understanding the spread of AMR as well as implications for the design of surveillance studies to capture their emergence.

## P375

# Contemporary syphilis is characterised by rapid global spread of pandemic lineages

Mathew Beale[1], Michael Marks[2], Michelle Cole[3], Min-Kuang Lee[4], Gwenda Hughes[3], Prenilla Naidu[5], Magnus Unemo[6], Mel Krajden[4], Sheila Lukehart[7], Muhammad Morshed[4], Helen Fifer[3], Nicholas Thomson[1]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]London School of Hygiene and Tropical Medicin, London, United Kingdom. [3]Public Health England, London, United Kingdom. [4]British Columbia Centre for Disease Control, Vancouver, Canada. [5]Alberta Precision Laboratories, Edmonton, Canada. [6]31WHO Collaborating Centre for Gonorrhoea and other Sexually Transmitted Infections, Department of Laboratory Medicine, Microbiology, Faculty of Medicine and Health, Örebro University, Örebro, Sweden. [7]University of Washington, Seattle, USA

## Abstract

Syphilis is a major sexually transmitted infection caused by the bacterium *Treponema pallidum* subspecies *pallidum*. The last two decades have seen syphilis prevalence rise in many high-income countries, yet the evolutionary and epidemiological drivers that underpin this are poorly understood, as is the global population structure. We assembled a geographically and temporally diverse collection of 726 *T. pallidum* genomes, and used detailed phylogenetic analysis and clustering to show that syphilis globally can be described by only two deeply branching clades. We show that both of these clades can be found circulating concurrently in 12 of the 23 countries sampled. To provide further phylodynamic resolution, we subdivided our genomes into distinct sublineages, and we show that sublineages in both major clades have expanded clonally across 9 countries. Moreover, pairwise genome analysis showed that recent isolates circulating in 14 different countries were genetically identical in their core genome to those from other countries, suggesting frequent exchange through international transmission pathways. This contrasts with the majority of samples collected prior to 1983, which are phylogenetically distinct from these more recently isolated sublineages. Bayesian temporal analysis provided evidence of a population bottleneck and decline occurring during the late 1990s, followed by a rapid population expansion a decade later. This was driven by the dominant *T. pallidum* sublineages circulating today, many of which are resistant to macrolides. Combined we show that the population of contemporary syphilis has undergone a recent and rapid global expansion.

## P376

# Genomic diversity of *Bacillus anthracis* in endemic settings: novel approaches and data

Taya Forde[1], Tristan Dennis[1], Ayesha Hassim[2], Ireen Kiwelu[3], Matej Medvecky[1], Blandina T. Mmbaga[3], Deogratius Mshanga[4], Henriette Van Heerden[2], Adeline Vogel[1], Ruth N. Zadoks[5], Tiziana Lembo[1], Roman Biek[1]

[1]University of Glasgow, Glasgow, United Kingdom. [2]University of Pretoria, Pretoria, South Africa. [3]Kilimanjaro Clinical Research Institute, Moshi, Tanzania, United Republic of. [4]Tanzania Veterinary Laboratory Agency, Arusha, Tanzania, United Republic of. [5]University of Sydney, Sydney, Australia

## Abstract

Anthrax, caused by the spore-forming bacterium *Bacillus anthracis*, remains endemic in many developing countries where it has significant impacts on the health and livelihoods of livestock-keeping communities. While the global genomic diversity of *B. anthracis* is well characterised, few studies have quantified its diversity in endemic settings at more local scales, where this information could be critical for elucidating transmission dynamics and guiding control efforts. We collected samples from 526 anthrax-suspected animal carcasses between 2016 and 2018 in the Ngorongoro Conservation Area in northern Tanzania. Seventy five percent were confirmed positive through qPCR, suggesting that anthrax is responsible for a large proportion of sudden deaths in livestock in this area. A subset of positive samples were cultured for whole genome sequencing (n = 73), including multiple isolates from individual carcasses. All sequenced isolates belonged to the Ancient A lineage of *B. anthracis*, a common strain in southeastern Africa. No clear spatial clustering was observed, possibly reflecting extensive animal movement related to local nomadic practices. Moreover, high levels of within-host diversity were observed which suggests that cases commonly result from simultaneous infection with multiple strains. Additionally, we trialed a targeted sequence capture approach on 93 samples. This was successful in recovering >80% of the chromosomal genome at > 15X coverage from 60% of samples tested, thus representing a valuable tool for culture-free sequencing of this high-risk bacterium. Our work paves the way for integrating genomic data for *B. anthracis* into epidemiological studies and monitoring of control programs in endemic areas worldwide.

**P377**

# Evolution of gene regulatory networks in a bacterial experimental system: Identifying general principles governing regulator rewiring from two contrasting mutational pathways

Matthew Shepherd[1], Aidan Pierce[1], Robert Jackson[2], Laurence Hurst[1], Tiffany Taylor[1]
[1]University of Bath, Bath, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom

## Abstract

The timing and magnitude of gene expression in living cells are carefully controlled by circuits of cis- and trans-regulatory elements called gene regulatory networks. Components of these networks can interact with non-cognate sites (i.e. they can act promiscuously), providing an evolutionary raw-material for repurposing or rewiring regulators to take on new functions. Here we investigate two distinct evolutionary pathways for repurposing transcription factors in a bacterial experimental system. A strain of the soil bacterium *Pseudomonas fluorescens* lacking the flagellum master regulator FleQ is challenged to rescue flagellar expression and motility. The primary mechanism observed to rescue motility is repurposing the nitrogen assimilation regulator NtrC, which occurs reliably and repeatability via a two step process. Through knockout of the *ntrC* gene, a secondary rarer mechanism was identified. Genomic and transcriptomic data indicates motility rescue through repurposing of the putative regulator PFLU1132, as well as the alginate biosynthesis regulator AlgB. Through transcriptomic analysis of these two pathways, two contrasting approaches to rescuing gene expression are observed. One involving targeted rewiring of the transcription factor, and the other involving global changes to gene expression to facilitate the promiscuity of several homologous regulators. Notably, we can provide empirical evidence for general principles constraining the evolution of transcription factors, including the expression of a regulator, and the structure of the gene regulatory network it is part of. Our work highlights the dynamic and complex functionality of transcription factors within control networks, and their ability to evolve and rewire to achieve new regulatory functions.

## P378

# Exploring bacterial diversity via a curated and searchable snapshot of archived DNA

Grace Blackwell[1,2], Martin Hunt[1,3], Leandro Lima[1], Kerri Malone[1], Gal Horesh[2], Blaise Alako[1], Zamin Iqbal[1], Nicholas Thomson[2,4]
[1]EMBL-EBI, Hinxton, United Kingdom. [2]Wellcome Sanger Institute, Hinxton, United Kingdom. [3]University of Oxford, Oxford, United Kingdom. [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

## Abstract

The open sharing of genomic data provides an incredibly rich resource for the study of bacterial evolution and function, and even anthropogenic perturbations such as the widespread use of antimicrobials. Whilst these archives are rich in data, considerable processing is required before a biological question can be addressed. Here, we have assembled, quality controlled and characterised 661,405 bacterial genomes that were in the European Nucleotide Archive (ENA) at the end of November of 2018, using a uniform standardised approach. A searchable index has been produced, facilitating the easy interrogation of the entire dataset for a specific gene or mutation. Our analysis shows how uneven the species composition is within this database, with just 20 of the total 2,336 species making up 90% of the high-quality genomes. The over-represented species tend to be acute/common human pathogens, often aligning with research priorities at different levels from individuals with targeted but focussed research questions, areas of focus for the funding bodies or national public health agencies, to those identified globally as priority pathogens by the WHO for their resistance to front- and last-line antimicrobials. Whilst this is a rich resource which often forms the context or references for multi-'omic' studies and supports discovery research in many domains, understanding the actual and potential biases in bacterial diversity depicted in this snapshot, and hence within the data being submitted to the public sequencing archives, is essential if we are to target and fill gaps in our understanding of the bacterial kingdom.

## P379

# In silico binding pocket analysis of antimicrobial peptide-chitosan nanosystem with serum proteases

Afreenish Hassan, Aamer Ikram
NUMS, Rawalpindi, Pakistan

## Abstract

Antimicrobial peptides (AMPs) have emerged as promising strategy which can offer robust bactericidal effect against multidrug resistant (MDR) bacteria. AMPs face instability due to presence of serum proteases. Computational studies help to understand the behavior of antimicrobial peptides with nano drug delivery systems.

In silico, circular ring using 10 monomers of chitosan and ionic crosslinker generated structure which is docked with mastoparan followed by molecular dynamic simulations (MDS) to check for interactions and stability, respectively. Binding pocket analysis is used to assess protease-peptide interface.

MDS for 100 ns shows stable hydrogen bonding between chitosan and mastoparan with binding energies of -3.6 and -7.4 kcal/mol. Volume and surface area of chitosan-mastoparan complex is found geometrically incompatible against binding pockets of various proteases.

Nanosystem provide safe and stable environment to antimicrobial peptides from proteolytic enzymes. The strong interaction between peptide and nanosystem may offer higher bactericidal effect against strains resistant to conventional antibiotics.

**P381**

# Establishing the fundamental principles of spore maturation in spores of human fungal pathogens

Megan McKeon, Christina Hull
University of Wisconsin-Madison, Madison, USA

## Abstract

Spores are a dormant, stress-resistant cell type used by fungi to spread to new environments. To survive, these cells must remain dormant until they encounter favorable conditions for germination, an essential differentiation process in which dormant spores transition into vegetatively growing cells. Despite the importance of spore germination in the life cycles of the majority of fungi, the molecular networks governing this fundamental process remain poorly understood in any system. Based on evidence that spores vary across populations, we hypothesized that dormant spore must undergo a maturation process in order to germinate efficiently. To test this hypothesis, we evaluated the molecular processes governing spore maturation and germination in the human fungal pathogen *Cryptococcus*. Using comparative transcriptomics of spores recovered from younger and older sexual crosses (3-day and 7-day), we identified a cohort of transcripts that are more highly represented in mature spores. This finding supports our hypothesis that the transcriptome of dormant spores is not static and that spores continue to mature after biogenesis. Transcripts enriched in mature spores are largely involved in nucleic acid and carbohydrate binding. The products of these transcripts may be important for facilitating rapid spores responses to nutrients in the external environment. We will continue to assess the roles of these candidate genes in spore maturation using gene deletion strains and high-resolution, quantitative germination assays. Together, these data establish the foundation for understanding the fundamental principles of spore maturation, dormancy, and germination.

## P382

# *Staphylococcus haemolyticus* as a reservoir of antimicrobial resistance genes in the preterm infant gut microbiome

Lisa Lamberte[1], Raymond Kiu[2], Robert Moran[1], William Rowe[1], Lindsay Hall[2,3], Willem van Schaik[1]
[1]University of Birmingham, Birmingham, United Kingdom. [2]The Quadram Institute, Norwich, United Kingdom. [3]Technical University of Munich, Munich, Germany

## Abstract

*Staphylococcus haemolyticus* is a clinically important and highly common opportunistic pathogen. It is particularly important in preterm infants as it is one of the leading causes of late-onset sepsis (LOS).

The success of *S. haemolyticus* in a clinical setting can be attributed to its involvement in the acquisition and dissemination of antimicrobial resistance. However, it is unclear whether horizontal gene transfer is also a contributor in the successful colonisation of *S. haemolyticus* in the gut microbiome of preterm infants. To address this, we isolated *S. haemolyticus* from stool samples of a cohort of preterm infants. The stool samples used in this study were collected as part of the Wellcome Trust-funded multi-centre BAMBI (Baby Associated MicroBiota of the Intestine) study. We then used a combination of genomics and experimental approaches to study the horizontal transfer of resistance genes identified from our library of 57 *S. haemolyticus* isolates. Phylogenetic reconstruction categorised our isolates into three distinct groups, with one major group consisting 39/57 (68%) of the isolates from the cohort. Additionally, whole genome sequencing analysis detected the presence of a functional aminoglycoside resistance gene, *aac(6')-aph(2")*, that is linked to *Tn*4001 and is widespread among the cohort.

Ultimately, results from these experiments show that antimicrobial resistance genes implicated in hospital-adapted *S. haemolyticus* isolates are also found within the gut microbiome of a cohort of preterm infants, and that *S. haemolyticus* is a reservoir for the spread of resistance genes

## P383

# Automated pseudogene detection reveals insights into historical gene sharing dynamics in prokaryotes

Nicholas J Dimonaco[1], Wayne Aubrey[1], Amanda Clare[1], Kim Kenobi[1], Chris Creevey[2]
[1]Aberystwyth University, Aberystwyth, United Kingdom. [2]Queen's University Belfast, Belfast, United Kingdom

## Abstract

In recent years it has become apparent that prokaryotic genomes contain large numbers of pseudogenised genes which may provide valuable insights into the recent functional history of an organism. However, pseudogenes are difficult to detect ab initio and are not routinely reported by gene prediction tools.

We present StORF-R (Stop-ORF-Reporter), a tool that takes as input an annotated genome and returns putative missed genes (functional and/or pseudogenised) from the intergenic regions. We show that this methodology can recover gene-families that the state-of-the-art methods continue to misreport or completely omit.

We applied StORF-R to the intergenic regions of 2,665 E. coli genomes and found on average 244 previously missed pseudogenised genes (with in-frame stop codons) per genome, many of which had high scoring similarity to known Swiss-Prot proteins. Many of these pseudogenised genes form widespread gene families across E. coli strains.

To investigate if this phenomenon exists in other taxa we further applied the methodology to 44,048 bacterial genomes representing 8,244 species from Ensembl. This revealed many gene-families spanning multiple species with large (>10,000) numbers of copies of both intact and pseudogenised versions. Many of these families had only previously been reported in a single or few genomes, though we detected many hundred pseudogenised versions with StORF-R, changing our understanding of how widespread these genes truly are.

These pseudogenised genes represent a pangenomic 'graveyard' which may alter our understanding of the definition of core and accessory genes for many species.

## P384

# No non-sense: the prevention of non-specific transcription in *Bacillus subtilis*

David Forrest, Emily Warman, David Grainger
Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom

## Abstract

RNA polymerase determines where to begin transcription when its σ factor recognises the AT rich -10 element of a promoter. Horizontally acquired DNA is AT rich, meaning these sequences are enriched for -10 elements. Therefore, horizontally acquired DNA sequences are hot spots for non-specific, spurious transcription. In the gram-negative organism *Escherichia coli*, toxic spurious transcription is prevented by the nucleoid protein H-NS (Histone-like nucleoid structuring protein) which forms long filaments along AT rich DNA. Whilst H-NS is not widely conserved, functionally analogous proteins are.

In the gram-positive organism *Bacillus subtilis*, Rok primarily functions as a key repressor of natural competence. Rok has also been observed binding across the genome at the promoters of genes within AT rich, horizontally acquired DNA. This preferential binding of AT rich DNA lead to Rok being classified as functionally analogous to H-NS.

To investigate spurious transcription in *B. subtilis*, we utilised transcriptomic techniques to determine transcription start sites in a Δ*rok* strain. In this deletion strain, the Rok regulon was upregulated. Unexpectedly, there was no increase in the number of transcription start sites, even in horizontally acquired DNA. This suggests that *B. subtilis* RNA polymerase uses other mechanisms for preventing non-specific transcription on AT rich DNA. Further *in vivo* and *in vitro* work point towards the major *B. subtilis* σ factor, $\sigma^A$, as being responsible for increased specificity. This is in stark contrast to *E. coli* and other bacteria, which depend on nucleoid proteins to prevent harmful, spurious transcription on AT rich, horizontally acquired DNA.

## P385

# *Ralstonia solanacearum* pangenome shows variable levels of genetic diversity within different geographic regions

Martina Stoycheva[1], Evie Farnham[1], Gaofei Jiang[2], Daniel Jeffares[1], Ville Friman[1]
[1]University of York, York, United Kingdom. [2]Nanjing Agricultural University, Nanjing, China

## Abstract

*Ralstonia solanacearum* species complex is an umbrella term for 3 bacterial phylogenetic species historically known as phylotypes. The bacteria are pathogens causing plant disease with characteristic wilting of the leaves across ~200 plant species. *R. solanacearum* has a tropical origin but has spread across the temperate and tropical regions of the world. Bacteria can survive in environmental reservoirs such as river water and soil making the disease hard to eradicate. Knowledge of this pathogen's ecology and evolution is insufficient despite of its economic significance. We investigated the pangenome of the *Ralstonia solanacearum* species complex using a global strain collection of 182 isolates from 6 different continents and 40 different hosts as well as two country specific sample sets: 176 isolates of agricultural and environmental reservoirs from England; and 95 agricultural isolates from China. The world collection represented isolates from all known phylotypes with core genome of 2,384 ortholog groups and an open pangenome of 16,500 showing the large diversity within the species complex. Notably, the whole English population was found to be exclusively from the Phylotype IIB sequevar 1 clonal complex (~10 core SNPs), despite ~30 years of persistence in the environment across the English river system. On the contrary, the population in China consisted of multiple different sequevars of phylotype I with high diversity in both core SNPs (~18,000) and accessory genes including key regulators controlling virulence. The factors limiting the diversity of these pathogens in some geographic locations and promoting higher diversity in others require further investigation.

## P386

# Covariation analysis of the master bacterial DNA replication initiator DnaA

Laura Dobby, Heath Murray
Newcastle University, Newcastle Upon Tyne, United Kingdom

## Abstract

Bacterial DNA replication initiation is governed by DnaA: an oligomeric protein belonging to the ATPases associated with diverse cellular activities (AAA+) superfamily. The master replication initiator is present in all known bacterial pathogens; thus, it is an attractive target for drug discovery. Here, an *in-silico* evolutionary covariation analysis of DnaA amino acid residues was conducted using EVcouplings, a Python framework, to investigate connections between key protein residues. Several co-evolving amino acid pairs were identified and characterized using PyMol to visualize the residues on DnaA crystal structures. I hypothesize that some co-evolving pairs, particularly those spatially connected, are likely important for structural integrity of the protein. Interestingly, one co-evolving pair of residues (*A. aeolicus* Val121 and Ser229) was found to be separated by ~27 angstroms, suggesting a more complex relationship connects these two sites. Thus, analysing co-evolution of DnaA residues supports known structural information and may predict either allosteric connections within DnaA or contacts between DnaA and other binding partners.

## P387

# Genome-wide discovery of pathogenicity loci in hyper-virulent *Streptococcus pneumoniae*

Chrispin Chaguza[1], Chinelo Ebruke[2], Madikay Senghore[3], Stephanie Lo[1], Peggy-Estelle Tientcheu[2], Rebecca Gladstone[1], Gerry Tonkin-Hill[1], Jennifer Cornick[4], Marie Yang[5], Archibald Worwui[2], Lesley McGee[6], Robert Breiman[7], Keith Klugman[7], Aras Kadioglu[5], Dean Everett[8], Grant Mackenzie[2], Anna Roca[2], Brenda Kwambana-Adams[9], Martin Antonio[2], Stephen Bentley[1]
[1]Parasites and Microbes Programme, Wellcome Sanger Institute, Cambridge, United Kingdom. [2]Medical Research Council (MRC) Unit The Gambia at the London School of Hygiene and Tropical Medicine, Fajara, Gambia. [3]Center for Communicable Disease Dynamics, Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, USA. [4]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi. [5]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [6]Respiratory Diseases Branch, Centers for Disease Control and Prevention, Atlanta, USA. [7]Rollins School of Public Health, Emory University, Atlanta, USA. [8]MRC Centre for Inflammation Research, Queens Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom. [9]14NIHR Global Health Research Unit on Mucosal Pathogens, Division of Infection and Immunity, University College London, London, United Kingdom

## Abstract

The propensity of *Streptococcus pneumoniae* serotypes to cause invasive diseases than asymptomatic carriage varies remarkably. However, the underlying genetic basis for serotype-specific pathogenicity of hyper-invasive pneumococci, particularly serotype 1, remains poorly understood as rare carriage makes population-level genomic comparisons of invasive and non-invasive strains challenging. Here, we investigate the contribution of bacterial genetics to the development of serotype 1 pneumococcal diseases endemic in Africa using a genome-wide association study. We show that genetic variants, mostly associated with insertion sequences, are significantly overrepresented among carriage than disease isolates ($P$=7.54×10$^{-24}$), suggesting a deleterious effect on strain invasiveness. The variants revealed high penetrance (≈100%) and heritability (≈95%), reflecting the proliferation of carriage-adapted clones and independent acquisitions through horizontal gene transfer. Furthermore, machine learning models distinguished infection status with ≈93% accuracy, underscoring the potential for predicting pathogenicity risk of clones through surveillance. Our findings highlight the impact of insertion sequences for pneumococcal pathogenicity and nasopharyngeal adaptation.

**P388**

# How Does Regulatory Evolution Differ Between Related Groups? Diverse, Repeatable, and Undiscovered Routes to Restore Motility Through Mutations in Gene Regulatory Networks in Two Immotile Bacterial Strains

Louise Flanagan, James Horton, Tiffany Taylor
University of Bath, Bath, United Kingdom

## Abstract

Gene regulatory networks are essential to organism survival as they allow rapid adaptation to changing environments. But how do these networks evolve? Removing a key component of a regulatory network can help us answer this. Deletion of the master regulator (*fleQ*) for flagellar synthesis genes renders *Pseudomonas fluorescens* strains (Pf0-1 and SBW25) immotile, but after being put under strong selection to swim, they reliably regain motility after a few days.

To uncover how the bacteria regain their motility, samples of cells were collected from the outermost edge of the motile zone on a swimming agar plate and then sequenced at a range of loci (previously shown to be key mutational sites).

In Pf0-1, a huge variety of mutations were observed across all candidate loci. In a complex nutrient environment (LB) 55% of restorative mutations occur in *glnA*, a component of the nitrogen regulatory pathway. In a minimal media environment (M9) 54% of these mutations occur in another nitrogen regulatory gene, *ntrB*. These findings contrast strongly with SBW25, where the same motility-restoring SNP (A289C *ntrB*) is repeatedly found across all nutrient environments. Interestingly, this SNP has never been seen in Pf0-1. When it is engineered into Pf0-1 however, swimming is restored.

These results highlight how preferential mutational routes can vary across environments, that not all viable routes may be uncovered, and that strain-to-strain differences can have a major impact on types of mutations that are uncovered and selected for by evolution.

## P389

# A Quantitative Genetic Interaction Map of HIV Infection

David Gordon[1], Ariane Watson[2], Assen Roguev[1], Simin Zheng[3], Gwendolyn Jang[1], Joshua Kane[4], Jiewei Xu[1], Jeffrey Guo[1], Erica Stevenson[1], Danielle Swaney[1], Kathy Franks-Skiba[1], Erik Verschueren[5], Michael Shales[1], David Crosby[1], Alan Frankel[1], Alexander Marson[1], Ivan Marazzi[3], Gerard Cagney[2], Nevan Krogan[1]
[1]University of California San Francisco, San Francisco, USA. [2]University College Dublin, Dublin, Ireland. [3]Icahn School of Medicine at Mount Sinai, New York, USA. [4]Nurix Therapeutics, San Francisco, USA. [5]Galapagos Inc., Mechelen, Belgium

## Abstract

Genome-wide genetic perturbation screens have enabled the systematic identification of individual genes impacting pathogen infection, however these single-gene perturbation studies do not provide contextual information for gene function, and may miss redundant pairs of genes. In contract, combinatorial genetic interaction screens reveal epistatic and synergistic relationships that inform hypothesis generation regarding gene function. Furthermore, when carried out at a large scale, genetic interaction mapping can identify gene clusters that correspond to protein complexes. Despite the power of this approach, large-scale genetic interaction mapping is largely unexplored for the study of pathogen infection.

We have developed a platform for quantitative genetic interaction mapping using viral infectivity as a functional readout and constructed a viral host-dependency epistasis map (vE-MAP) of 356 human genes linked to HIV function, comprising >63,000 pairwise genetic perturbations. The vE-MAP provides an expansive view of the genetic dependencies underlying HIV infection and can be used to identify drug targets and study viral mutations. We found that the RNA deadenylase complex, CNOT, is a central player in the vE-MAP and show that knockout of CNOT1, 10, and 11 suppressed HIV infection in primary T cells by upregulating innate immunity pathways. This phenotype was rescued by deletion of IRF7, a transcription factor regulating interferon-stimulated genes, revealing a previously unrecognized host signaling pathway involved in HIV infection. The vE-MAP represents a generic platform that can be used to study the global effects of how different pathogens hijack and rewire the host during infection.

## P390

# The Two N-terminal Thioredoxin Domains of Protein Disulfide Isomerase are Sufficient for Cholera Toxin Disassembly

Antonio Mele, Antonio Torres, David Delgado, Ken Teter
University of Central Florida, Orlando, USA

## Abstract

Cholera is an infection due to ingestion of water containing *Vibrio cholerae* from fecal contamination. Cholera toxin (CT), a virulence factor released by *V. cholerae*, is comprised of two "A" subunits, CTA1 and CTA2, and a B pentamer. CT binds to GM1 on the surface of an infected individual's cells and is internalized for delivery to the endoplasmic reticulum (ER) via vesicle carriers. In the ER, the disulfide bond linking CTA1 to CTA2 is reduced and protein disulfide isomerase (PDI) dislodges CTA1 from the rest of the toxin, allowing it to enter the cytosol. CTA1 then engages in a signal cascade that ultimately results in a diarrheal response.

PDI has four thioredoxin-like domains, but it is currently unknown which domains contribute to the disassembly of CT. The objective of this project was to understand the structural basis for CT disassembly by PDI. To achieve this goal, PDI deletion constructs containing various subsets of the domains were purified and run in an ELISA disassembly assay using plates coated with GM1 and CT. It was found that full-length PDI disassembled 11-13% of CT in the ELISA plate, and the two N-terminal thioredoxin-like domains were as efficient at CT disassembly as full-length PDI.

These findings suggest that full-length PDI is not necessary for disassembling CT. Even an incomplete deletion construct can disassemble CT with comparable efficiency. This knowledge will lead to further experimentation that could, in the future, lead to the development of cholera treatments and preventatives.

## P391

# Development of Hybridised Cathelicidin and Human Beta-Defensin as Novel Treatment for Gram-Positive Bacterial Keratitis

Darren Ting[1], Roger Beuerman[2], Rajamani Lakshminarayanan[2], Imran Mohammed[1], Harminder Dua[1]
[1]University of Nottingham, Nottingham, United Kingdom. [2]Singapore Eye Research Insitute, Singapore, Singapore

## Abstract

**Purpose:** To generate potent hybridized human-derived host defense peptides (HDPs) as novel topical antimicrobial therapy for bacterial keratitis (BK).

**Methods:** Hybrid peptides were rationally engineered through combination of functional amino acids in parent HDPs, including LL-37 and human beta-defensin (HBD)-1 to -3. Minimal inhibitory concentration (MIC) and time-kill assays were performed to determine the efficacy of peptides, and cytotoxicity was evaluated against human corneal epithelial cells and erythrocytes. SYTOX green assay and molecular dynamic (MD) simulation were performed to examine the underlying mechanism of action. In vivo safety and efficacy of the most promising peptide was examined in the murine corneal wound healing and *Staphylococcus aureus* (ATCC SA29213) keratitis models, respectively.

**Results:** A second-generation hybrid peptide (HDP23) demonstrated good efficacy against methicillin-sensitive and methicillin-resistant *S. aureus* (MIC=5.2-10.4mM or 12.5-25.0mg/ml) and *P. aeruginosa* (MIC=20.9mM or 50mg/ml). HDP23 (2x MIC) killed all the bacteria within 30 mins, which was 8 times faster than amikacin (20x MIC). At 200mg/ml (16x MIC), HDP23 was shown to be relatively safe against HCE-2 (<30% toxicity) and erythrocytes (<10% toxicity). The antibacterial mechanism of action of HDP23 was likely attributed to its membrane-permeabilising activity, evidenced by the significant (60%) uptake of SYTOX green and MD simulation. Pre-clinical murine studies showed that HDP23 0.05% (500mg/ml) achieved a median reduction of *S. aureus* bacterial viability by 94% (or 1.2 $\log_{10}$ CFU/ml) while not impeding corneal healing.

**Conclusions:** Rational modification of human-derived HDPs has led to the generation of a potentially efficacious topical antimicrobial agent for treating Gram-positive BK.

## P392

# Genotypic and phenotypic virulence analysis of Salmonella Dublin isolates associated with bovine abortion.

Jemma Franklin[1], Adam Blanchard[1], Gail Wise[2], Sabine Totemeyer[1]
[1]University of Nottingham, Nottingham, United Kingdom. [2]Animal and Plant Health Agency, Surrey, United Kingdom

## Abstract

*Salmonella enterica* serovar Dublin is one of the most common bacterial causes of bovine abortion in the UK. Despite its prevalence, little is known about how the disease progresses from initial infection to abortion. *S*. Dublin is known to colonise the bovine placentome, formed of maternal caruncular and fetal cotyledonary tissues. *S.* Dublin virulence is dependent upon Type 3 Secretion Systems encoded for on *Salmonella* Pathogenicity Islands -1 and -2. Investigating these genetic regions may give rise to a greater understanding of *S.* Dublins pathogenicity, and may identify genes associated with abortion.

The presence of virulence genes in 15 *S.* Dublin isolates from UK bovine abortions in 2017 and a reference isolate was investigated. Following Illumina Short Read Sequencing and genome assembly, virulence genes with 90% or higher identity were identified. Whole genome sequence and virulence profiles of these isolates were compared to a library of 250 UK isolates, selected on the availability of metadata describing where they were isolated from. UK abortion isolates from this study clustered predominantly with *S.* Dublin isolates from farm environments, whilst the majority of isolates associated with human disease and food cluster separately.

The ability of the abortion-associated isolates to infect and replicated within Bovine Caruncular Epithelial Cells (BCECs) was investigated as a marker of phenotypic virulence. BCECs were infected with multiplicities of infection of 1 and 10 at 2 and 24h, and the bacteria were recovered. All isolates were able to invade and replicate in BCECs which mirrored their similarity in virulence profiles.

## P394

# A study on the antibacterial efficacy of Halohydantoin-containing foams

Andreea-Gabriela Nedelea[1], Lori Robins[2], Jeffrey Williams[3], Sarah Maddocks[1]
[1]Cardiff Metropolitan University, Cardiff, United Kingdom. [2]University of Washington, Bothell, Bothell, USA. [3]Medesol Inc, Bellevue, USA

## Abstract

Heterocyclic N-halamine compounds such as 1-monochloro-5,5-dimethylhydantoin (MCDMH) and 1,3-dichlor-5,5-dimethylhydantoin (DCDMH) show antimicrobial activity and odour control. Both MCDMH and DCDMH stabilise and display oxidative chlorine (Cl+1) and, in aqueous environments, generate hypochlorous acid (HOCl). It is well known that HOCl, produced by neutrophils as part of the respiratory immune response, is used as a defence mechanism against foreign bodies. This study aimed to investigate the antibacterial efficacy of proprietary foaming formulations containing mixtures of MCDMH and DCDMH provided by MedeSol Inc., as a potential delivery mechanism for HOCl. The efficacy of the foams containing active chlorine concentrations of 350 ppm and 1050 ppm was tested against single and multi-species bacterial mixtures of *S. aureus*, *P. aeruginosa*, *E. coli*, *E. faecalis* and *K. pneumoniae*. Cellulose filters were contaminated with single/multispecies bacterial suspensions and left to air-dry; the two foam concentrations were applied for 15, 30 and 60 seconds at a time. Samples were taken and the total viable count method was employed to determine antibacterial efficacy of the foams. Following the use of both foam concentrations, no colonies were recovered at any time-points in both single and multi-species experiments. In contrast, the negative controls where the foam application was omitted, resulted in high numbers of bacterial colonies. MCDMH-containing foams have very strong antibacterial potential as a result of active chlorine release. Further studies are needed to determine concentration dependence and use in other applications including hand sanitation.

**P395**

# Assessing the interspecies relationship between *Pseudomonas aeruginosa* and *Staphylococcus aureus* in mixed biofilms grown in alginate beads and collagen scaffolds.

Ammara Khalid, Michael Beeton, Sarah Maddocks
Cardiff Metropolitan University, Cardiff, United Kingdom

## Abstract

Polymicrobial biofilms in chronic infected wounds harbour different bacterial species that interact with each other, competing or co-operating to survive. The two most common pathogens co-isolated from chronic wound biofilms are *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Evidence from *in vitro* biofilms models have shown these two bacteria interact and data suggests that *P. aeruginosa* inhibits the growth of *S. aureus* in mixed biofilms. This study aimed to assess the growth of these two species in a complex polymicrobial biofilm in a 3D matrix comprised of either alginate (1.5% w/v) or a collagen scaffold.

Using a five-species biofilm (*S. aureus, P. aeruginosa, Citrobacter freundii, Enterococcus faecalis* and *Escherichia coli*), with all bacteria inoculated at time zero, it was consistently observed that *P. aeruginosa* was not recoverable over a 72h period, with sampling every 24h. However, *P. aeruginosa* grew well if it was added to a pre-formed four-species biofilm. Further, *P. aeruginosa* was seen to inhibit the growth of *S. aureus* after 24h subsequent co-culture in the pre-formed biofilm, which resulted in the emergence of small colony variants of *S. aureus*. Interestingly when *P. aeruginosa* was co-inoculated in a four species biofilm that did not contain *S. aureus,* its growth was not inhibited, suggesting a competitive interaction between these two bacteria during establishment of the early biofilm. These data were consistent in alginate beads and collagen scaffolds. In a chronic wound *P. aeruginosa* is regarded as a late coloniser and the phenomena observed in this study might be reflective of this.

**P396**

# Antimicrobial potentials of *Lantana camara montevidensis* leaf extract on wounds infected with *Candida* isolates using animal models

OFONIME OGBA, SUNDAY EDIM
UNIVERSITY OF CALABAR, CALABAR, Nigeria

## Abstract

**Background:** There is an increased focus in the development of antimicrobials of herbs origin because they possess active chemotherapeutic effect on different kinds of infectious diseases. Many communities in Nigeria and other African countries use plants to treat various infections, including wounds. New antimicrobial agents are being developed in response to the emergence of resistance to existing antibiotics and antifungal agents. *Lantana camara* leaves are easily accessible at low or no cost in resource poor communities. The aim of this study was to determine the antimicrobial activity of *Lantana camara* leaf extract against selected *Candida* isolates from infected wounds invitro and in vivo using animal models. **Methods:** Aqueous and methanol leaf extraction was done using Soxhlet apparatus. Ten *Candida* isolates associated with wound infections were re-identified and used for the study. Antifungal susceptibility testing was done using the E-Test strips. Fifteen healthy male Wistar rats were used for the study. The rats were anesthetized before making incision wounds on the neck region. The rats were treated with 100mg/ml, 50mg/ml and 25mg/ml concentration of extract topically. The skin tissues of the sacrificed rats were obtained for histological examination using Heamatoxylin and eosin technique. **Results:** The susceptibility rate of the *Candida* isolates ranged from (0.0% - 40.0%). Fluconazole was the most effective antifungal. Isolates were most susceptible (40.0%) to 100mg/ml concentration of extract. Rats treated with 100mg/ml methanol extract had significant mean wound contractions of 70.33±0.58 with damaged tissue repair. **Conclusion:** Methanol extract of *Lantana camara* leaf has antimicrobial activity on *Candida* isolates and topical healing effect on *Candida*

## P397

# Role of enteroaggregative *E.coli* induced activation of epidermal growth factor receptor in cultured human intestinal epithelial cell lines

Archana Joon, Shipra Chandel, Sujata Ghosh
Post Graduate Institute of Medical Education & Research, Chandigarh, India

## Abstract

Enteric pathogens exploit the versatility of cytoskeletal system for internalization into non-phagocytic cells, as a crucial step in their pathogenic life cycle. Enteropathogenic *Escherichia coli* (EPEC) and Enterohemorrhagic *Escherichia coli* (EHEC) were shown to form actin pedestals in host cells using type-III secretion system. Earlier, we reported that EAEC induced increase in intracellular calcium ions might have crucial role in F-actin rearrangements in INT-407 cells leading to its invasion. It was suggested that EGFR might contribute in Rck-mediated adherence and invasion of *Salmonella* in host cells. In the present study, we assessed the role of EAEC induced activated EGFR in human intestinal epithelial cell lines (INT-407 & HCT-15). The presence of activated EGFR was detected in membrane fractions of each cell line, infected with two different strains of EAEC (EAEC-T8 & EAEC-O42) separately for 3h in presence and absence of Tyrphostin AG1478 (EGFR-inhibitor), by western immunoblotting using p-EGFR (Y1068) antibody. Adherence and invasion of EAEC-T8 to each cell line were checked in presence of Tyrphostin AG1478. Further, the effect of Tyrphostin AG1478 on cytoskeletal F-actin rearrangement of EAEC-T8 infected cells was assessed under confocal microscope following staining with TRITC-phalloidin. EAEC-T8 induced maximum increase in EGFR autophosphorylation at Y1068. Adherence and invasion of EAEC-T8 as well as this organism induced cytoskeletal F-actin polymerization were found to be inhibited in presence of Tyrphostin AG1478. Our study revealed that EAEC induced activated EGFR might play a major role in host cell adherence and cytoskeletal rearrangements leading to invasion of the organism in these cells.

## P398

# The occurrence of *Toxoplasma gondii* on raw leafy Vegetables in Gaza – Palestine

Zuhair DARDONA[1], Adnan AL HINDI[2], Mohamed Hafidi[3], Ali BOUMEZZOUGH[3], Samia BOUSSAA[3,4]
[1]2. Governmental Medical Services, Gaza, Gaza, Palestine. [2]Medical Laboratory Sciences Department, Faculty of Health Sciences, Islamic University of Gaza,, Gaza, Palestine. [3]Microbial Biotechnologies, Agrosciences and Environment Laboratory (BioMAgE), Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakesh, Morocco. [4]4. ISPITS-Higher Institute of Nursing and Health Techniques, Ministry of Health,, Marrakesh, Morocco

## Abstract

The present study was carried out to investigate the occurrence of *T. gondii* oocyst in local leafy vegetables. For this purpose, fifty samples of each kind of six different leafy plants sold in markets, supermarkets, and retail sellers were randomly collected during the period from March to August 2019. The samples were examined microscopically and confirmed using the PCR method, only 19 out of the 300 samples (6.33%) were found to be contaminated, whereas by using Sheather's solution method, 35 out of the 300 samples (11.66%) were contaminated. Among the six various plants and according to the PCR method, mint held the highest rate of contamination (10.00%), followed by both watercress and dill with a similar percentage (8.00%), parsley (6.00%), thyme (4.00%), and finally, lettuce carrying the lowest rate (2.00%). Even though the sequence among those contaminated plants was found similar in the PCR and the Sheather's solution, the rates were different. With this method, mint stood at (18.00%), watercress (14.00%), dill (13.00%), parsley (10.00%), thyme (10.00%) and lettuce (6.00%). Moreover, the present study has reported that, the relationship between the period of samples collection and *T. gondii* contamination is statistically significant. Whereby, the highest rate of contamination recorded was in July, followed by June, then August. Based on the findings of the present study, leafy vegetables are quite vulnerable to *T. gondii* contamination, particularly mint. This study recommends that, identifying leafy vegetables as a potential risk factor in transmitting *T. gondii* to humans in Gaza, Palestine.

## P400

# Atypical presentation of Lemierre's syndrome

Diviyesh Panchani, Deepak Nair
Glan Clwyd Hospital, Rhyl, United Kingdom

### Abstract

65-year-old gentleman who was previously fit and well with no known medical co-morbidities was admitted with symptoms of dysuria, urinary frequency, pyrexia, chills and lethargy. His vitals showed he was hypotensive, tachycardiac and pyrexial. Urine dip was heavily positive for leukocytes and nitrites. He had markedly elevated inflammatory markers and mild AKI based on his bloods. He was commenced on the urosepsis pathway and initiated on Tazocin.

On day two he complained of severe left-sided neck pain with reduced range of motion, denied any sore throat. Examination of neck and oropharynx was thus was prescribed simple analgesia.

Microbiology grew Gram -ve bacilli and Metronidazole was added with stat dose of Gentamycin as per microbiologist advise. Urine dip was negative for culture and ultrasound KUB was normal.

On Day 3 he developed severe neck swelling, erythema and reduced lateral flexion of the neck. Examination showed vague swelling of the neck with ill-defined borders. Ultrasound of neck showed abscess collection around the Internal Jugular Vein (IJV) with anaechoic contents within the IJV. Subsequent CT confirmed a 7cm Left IJV thrombosis. Final microbiology culture grew Fusobacterium Necrophorium.

A diagnosis of **Lemierre's syndrome** was made. Patient was continued on IV Tazocin and Metronidazole as per sensitivities and later switched to oral metronidazole. He was initiated on Warfarin anticoagulation. He made a remarkable recovery and was discharged with outpatient ENT and haematology follow-up.

The unique nature of this case was its absurdly atypical presentation and rarity of the disease making the diagnosis even more challenging

## P401

# *Klebsiella*-macrophage arms-race: dissecting how the pathogen copes with a macrophage lifestyle

Brenda Morris, Amy Dumigan, Joana Sa Pessoa, Jose Bengoechea
Wellcome Wolfson Institute for Experimental Medicine, Belfast, United Kingdom

## Abstract

*Klebsiella pneumoniae* is a multi-drug resistant human pathogen causing urinary tract infections, pneumonia and septicaemia. In a landmark contribution from our laboratory, we showed that *K. pneumoniae* persists intracellularly within the *Klebsiella* containing vacuole (KCV), by blocking phagolysosomal maturation. In this work, we have investigated the metabolic adaptation of *K. pneumoniae* to an intracellular lifestyle. *In vitro*, *Klebsiella* is capable of carrying out glycolysis/gluconeogenesis, the pentose phosphate pathway, the citric acid cycle (TCA) and glyoxylate pathway. To dissect intracellular *K. pneumoniae* metabolism, we have followed a genetic approach, generating mutants in key enzymes of each pathway. These mutants were assessed for *in vitro* growth kinetics using different carbon sources, adhesion, phagocytosis and intracellular survival in macrophages, as well as intracellular trafficking and macrophage response to infection. Also, using a pharmacologic approach and real time flux analysis, we have determined macrophage metabolism upon *Klebsiella* infection, and linked it to intracellular survival and immune responses. Our data revealed two categories of *K. pneumoniae* metabolic mutants with defects in intracellular survival. One set, in which the *Klebsiella*-controlled blockage of phagolysosome maturation was compromised, and the other unable to survive in the KCV. The former set also elicited a macrophage inflammatory response. Furthermore, *Klebsiella* infection induced an increase in macrophage glycolysis. Pharmacologic inhibition of this glycolysis and of TCA negatively impacted *Klebsiella* intracellular survival. In conclusion, we have uncovered new means exploited by a human pathogen to subvert macrophage intrinsic immunity, and discovered that *K. pneumoniae* rewires macrophage metabolism to persist intracellularly.

**P402**

# Investigating intestinal infection by *Mycobacterium avium* ssp *paratuberculosis* and the role of adhesins in this process using a bovine enteroid system

Rosemary Blake, Jayne Hope, Jo Stevens
The Roslin Institute, Edinburgh, United Kingdom

## Abstract

The current control measures for Johne's disease (JD), caused by *Mycobacterium avium* ssp *paratuberculosis* (MAP), are ineffective and do not reduce spreading of the disease or infection. Better diagnostic tools and vaccine targets must be identified if we hope to mitigate the impact of JD on the economy and improve animal welfare. We believe that by investigating the early interaction of MAP with the host at a cellular and molecular level, new diagnostic markers and vaccine targets will be identified. Due to the slow-growing nature of MAP and its long subclinical period, studying the infection *in vivo* is difficult. Therefore, we aim to study MAP infection in cattle using a bovine intestinal organoid (enteroid) system which is reproducible and physiologically relevant. To this end, we have generated and characterised bovine enteroids, and 2D-monolayers from these enteroids, using RT-PCR and antibody staining. The virulence of different strains of MAP can be assessed with this model, which is of particular interest to compare the lab-adapted reference strain, K10, to a recent field isolate that has been sequenced in our lab, C49. Adhesins expressed on the surface of MAP are critical for the initial host cell tropism displayed by MAP. Here, we have studied the role of a subset of MAP mammalian-cell-entry (*mce*) genes using a non-invasive *E. coli* expression system to enhance our understanding of the bacterial molecules required in the earliest steps in host cell infection. This will inform the development of future targeted approaches for the inhibition of MAP infection.

**P403**

# Decreased mortality in patients with bloodstream infections caused by multidrug resistant *Escherichia coli*

Maria Getino[1], Peter Riley[2,3], David Baker[4], Anuradha Ravi[4], Arnoud van Vliet[1], Mark Pallen[4,1,5], Roberto La Ragione[1]
[1]University of Surrey, Guildford, United Kingdom. [2]St George's University Hospitals NHS Foundation Trust, London, United Kingdom. [3]St George's, University of London, London, United Kingdom. [4]Quadram Institute Bioscience, Norwich, United Kingdom. [5]University of East Anglia, Norwich, United Kingdom

## Abstract

Bloodstream infections, especially those resistant to antibiotics, are on the rise. *Escherichia coli* is the most common microorganism implicated in these serious infections and multidrug resistant isolates are frequently isolated. However, there remains a paucity of data in the literature relating to the risk factors associated with these infections. Therefore, a 12-month longitudinal study was conducted to decipher potential risk factors. The study integrated genomic and clinical characteristics of a panel of 111 *E. coli* isolates recovered from the blood of patients at a South London UK hospital between 2017 and 2018.

Most bloodstream infections were community-acquired and derived from a urinary tract source, mainly affecting patients over 65 years of age. Antibiotic resistance incidence varied significantly throughout the year and was associated with previous antibiotic treatment and specific sources of infection, especially intra-abdominal. ST131 was the most common multi-locus sequence type, frequently harbouring antibiotic resistance genes and plasmids, and the presence of IncFI plasmids was associated with isolates being multidrug resistant.

All-cause 30-day mortality was significantly associated with the source of infection and, although not significantly, mortality was higher than the 13% average in patients with isolates belonging to ST131 and ST69, in age groups of 49-64 and 79-98, with hospital-acquired infections, male patients and, unexpectedly, with non-multidrug resistant isolates.

This study highlights the need of integrating molecular and clinical data at a larger scale to better understand the main factors driving mortality in patients with bloodstream infections and inform the design of effective interventions.

## P404

# Elucidating the Mechanisms of eDNA Release within *S. gordonii* Biofilm

Hannah Serrage[1], Angela Nobbs[1], Mark Jepson[1], Domininc Alibhai[1], Stephen Cross[1], Nadia Rostami[2], Nicholas Jakubovics[2]
[1]University of Bristol, Bristol, United Kingdom. [2]Newcastle University, Newcastle, United Kingdom

## Abstract

**Background** *Streptococcus gordonii* is a ubiquitous constituent of dental plaque biofilm. Extracellular DNA (eDNA) is an important component of plaque that maintains biofilm structural integrity. However, little is understood about eDNA release mechanisms, in part due to a lack of reliable methods of eDNA visualisation and quantification. This project aimed to exploit a high-throughput image analysis technique to quantify eDNA and to use this to assess the role of peptide signalling systems (ComCDE, Hpp) in mediating eDNA release within *S. gordonii* biofilm.

**Methods** Biofilms of wild-type *S. gordonii* or ComCDE/Hpp knockout mutants were formed ± competence stimulating peptide (CSP). Levels of eDNA strands, immunolabelled with Alexafluor594®, were then quantified by widefield microscopy using the modular image analysis plugin on Fiji. Total biomass was assessed by crystal violet stain.

**Results** Levels of eDNA were significantly reduced relative to wild-type in the absence of the ComCDE or Hpp systems. Moreover, this deficiency could be rescued by application of exogenous CSP to the Δ*comCDE* or Δ*hppH* mutants, but not the dual Δ*comCDE*/*hppH* mutant. This effect was not seen with a scrambled version of CSP.

**Conclusion** Taken together, these data imply that CSP can modulate eDNA release within *S. gordonii* biofilms. Moreover, this mechanism is not exclusively mediated by the cognate ComCDE system but exploits cross-talk with the Hpp hexapeptide permease system. Greater insights into eDNA release mechanisms such as these for oral microbes could potentially enable eDNA to be targeted for the management of dental plaque biofilm to promote oral health.

## P405

# Molecular and cellular insight into Escherichia coli SslE and its role during biofilm maturation

Paula Corsini[1], Sunjun Wang[1], Saima Rehman[1], Katherine Fenn[2], Amin Sagar[3], Slobodan Sirovica[4], Leanne Cleaver[1], Charlotte Edwards-Gayle[5], Giulia Mastroianni[4], Ben Dorgan[1], Lee Sewell[1], Steven Lynham[1], Dinu Iuga[6], Trent Franks[6], James Jarvis[1], Guy Carpenter[1], Michael Curtis[1], Pau Bernado[3], Vidya Darbari[4], James Garnett[1]

[1]King's College London, London, United Kingdom. [2]Queen Mary University of London, Lon, United Kingdom. [3]Centre de Biologie Structurale, Montpellier, France. [4]Queen Mary University of London, London, United Kingdom. [5]Diamond Light Source, Didcot, United Kingdom. [6]University of Warwick, Warwick, United Kingdom

## Abstract

*Escherichia coli* is a Gram-negative bacterium that colonizes the human intestine and virulent strains can cause severe diarrhoeal and extraintestinal diseases. The protein SslE is secreted by a range of pathogenic and some commensal *E. coli* strains. It can degrade mucins in the intestine, promotes biofilm maturation and in virulent strains, it is a major determinant of infection, although how it carries out these functions is not well understood. Here we examine SslE from the *E. coli* Waksman and H10407 strains and using electron microscopy (EM), small angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR) spectroscopy and biochemical analyses we show that SslE has a highly dynamic structure in solution. We also directly observe acidification within mature biofilms, describe a mechanism where SslE forms unique functional fibres under these conditions and determine that these SslE aggregates can bind cellulose, a major exopolysaccharide of many *E. coli* biofilms. Our data indicates that the spatial organization of SslE polymers and local pH are critical for biofilm maturation and SslE is a key factor that drives persistence of SslE-secreting bacteria during acidic stress.

## P406

# Histopathological analysis of placental lesions caused by Chlamydia abortus 1B vaccine strain in vaccinated ewes.

Sergio Gastón Caspe[1,2,3], Morag Livingstone[1], Sean Wattegedera[1], Kevin Aitchison[1], Clare Underwood[1], Elspeth Milne[2], Neil Sargison[2], Francesca Chianini[1], Javier Palarea-Albaladejo[4], David Longbottom[1]
[1]Moredun Research Institute, Edinburgh, United Kingdom. [2]Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom. [3]Instituto Nacional de Tecnologia Agropecuaria, Mercedes, Corrientes, Argentina. [4]Biomathematics & Statistics Scotland, Edinburgh, United Kingdom

## Abstract

### Background

*Chlamydia abortus* is one of the most diagnosed causes of infectious abortion in small ruminants. Infections can be controlled using the live, attenuated *C. abortus* strain 1B vaccine, which has been associated with infection and abortion in animals. This study aimed to compare the severity and the distribution of lesions caused by this vaccine strain (vt) with those resulting from a wild-type (wt) infection.

### Methods

Two grossly affected and 1B-positive (by qPCR and RFLP analysis) placentas from a vaccinated sheep flock were analysed. Histopathological lesions and immunohistochemical labelling (IHC) were graded (increasing score from 0 to 5) according to their severity and distribution. Pathology in the vt infected placentas was compared with that in two wt infected placentas. Datasets generated for observed histological and pathological features were analysed using principal component analysis (PCA).

### Results

Histopathologically, the lesions in both vt and wt-placentas presented as typical multifocal necrosuppurative placentitis, associated with vasculitis, mural necrosis, and thrombosis. IHC for *C. abortus* revealed intense staining with a multifocal distribution in most cotyledons in both vt and wt placentas. Comparison of the pathological lesions between vt and wt by PCA revealed a similar distribution and severity, revealing a strong association with features such as necrosis and inflammatory infiltration between vt and wt placentas. A weaker association with IHC was observed.

### Conclusion

This study shows that the *C. abortus* attenuated 1B vaccine strain presents no significant differences in severity and distribution of pathological lesions from those typically observed following a wt infection.

## P408

# Heterogenous susceptibility to R-pyocins in populations of *Pseudomonas aeruginosa* sourced from cystic fibrosis lungs

Madeline Mei, Jacob Thomas, Stephen Diggle
Georgia Institute of Technology, Atlanta, USA

## Abstract

Bacteriocins are proteinaceous antimicrobials produced by bacteria which are active against other strains of the same species. R-type pyocins are phage tail-like bacteriocins produced by *Pseudomonas aeruginosa*. Due to their anti-pseudomonal activity, R-pyocins have potential as therapeutics in infection. *P. aeruginosa* is a Gram-negative opportunistic pathogen and is particularly problematic for individuals with cystic fibrosis (CF). *P. aeruginosa* from CF lung infections develop increasing resistance to antibiotics, making new treatment approaches essential. *P. aeruginosa* populations become phenotypically and genotypically diverse during infection, however, little is known of the efficacy of R-pyocins against heterogeneous populations. R-pyocins vary by subtype (R1-R5), distinguished by binding to different residues on the lipopolysaccharide (LPS). Each type varies in killing spectrum, and each strain produces only one R-type. To evaluate the prevalence of different R-types, we screened *P. aeruginosa* strains from the International Pseudomonas Consortium Database (IPCD) and from our biobank of CF strains. We found that (i) R1-types were the most prevalent R-type among strains from respiratory sources; (ii) there are a large number of strains lacking R-pyocin genes, and (iii) isolates collected from the same patient have the same R-type. We then assessed the impact of diversity on R-pyocin susceptibility and found a heterogenous response to R-pyocins within populations, likely due to differences in the LPS core. Our work reveals that heterogeneous populations of microbes exhibit variable susceptibility to R-pyocins and highlights that there is likely heterogeneity in response to other types of LPS-binding antimicrobials, including phage.

**P410**

# Novel Primers for the Detection and Differentiation of *S. zooepidemicus* and *S. equi* by PCR

Tanya Mikaiel, Judy Mitchell, Jackie Cardwell, Simon Priestnall
RVC, London, United Kingdom

## Abstract

*Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) is a commensal opportunistic bacterium associated with outbreaks of equine respiratory disease alongside a diverse range of diseases in different species. The closely-related *Streptococcus equi* subspecies *equi* (*S. equi*) is the causative agent of strangles, the most frequently diagnosed highly contagious equine disease. Despite differing clinical signs, the two subspecies show 97% DNA homology and respectively present serious equine health and welfare concerns.

Rapid diagnostics are fundamental in the control of *S. zooepidemicus* and *S. equi.* Routine diagnostics predominantly utilise bacterial culture, which has a lengthy turnaround time. Polymerase chain reaction (PCR), such as that referenced in Alber *et al.*, (2004), which amplifies two targets, one at species level followed by a *S. equi* specific gene, offers a widely used and faster alternative.

Currently there are no PCR assay targeting a unique region of *S. zooepidemicus* and therefore testing is not independent from *S. equi* screening. By utilising the species-specific gene *sodA,* and *SeeH* and *LacD* genes unique to *S. zooepidemicus* and *S. equi* respectively, a rapid, multiplex PCR was developed and validated. Nucleic acid from isolates and clinical samples were tested, demonstrating the assay to be sensitive and specific. The assay provides a rapid, reliable and economical solution to the identification and differentiation of both *S. zooepidemicus* and *S. equi*.

## P411

# Non-typeable *Haemophilus influenzae* biofilm formation on primary human airway epithelia cultured at air-liquid interface

Katie L Horton[1,2], Janice L Coles[1,2], Jana F Hueppe[1,2], David W Cleary[1,2], Claire L Jackson[1,2], Jane S Lucas[1,2], Raymond N Allan[3]
[1]School of Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom. [2]NIHR Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom. [3]Leicester School of Pharmacy, Faculty of Health and Life Sciences, De Montfort University, Leicester, United Kingdom

## Abstract

### Introduction

Primary ciliary dyskinesia (PCD) is an inherited heterogeneous disorder associated with defective motile cilia on airway epithelia, resulting in impaired mucociliary function and ultimately, bronchiectasis. Non-typeable Haemophilus influenzae (NTHi) is frequently isolated from PCD airways where it resides in biofilms. It is unclear if biological pathways in response to NTHi infection differ in PCD, contributing to increased susceptibility to biofilm-associated infection.

### Aim

The development of a biologically representative co-culture model to investigate the interactions between PCD airway epithelia and NTHi biofilms.

### Methods

Air-liquid interface (ALI)-cultured non-PCD primary nasal cells were grown in transwells. Barrier properties were assessed by trans-epithelial electrical resistance (TEER), FITC-dextran passage and tight junction staining. Ciliary function was assessed by high-speed video microscopy at 37˚C. ALI cultures were infected with a PCD NTHi isolate at multiplicity of infections (MOIs) 10, 50 or 100 and co-cultured to form biofilms over 3 days. TEER was measured pre- and post-infection, NTHi recoverability assessed by conventional culture, and biofilm formation confirmed by scanning electron microscopy (SEM).

### Results

Epithelial culture at ALI was successful, with tight barriers being formed and normal ciliary beat pattern and frequency recorded (mean 14Hz). Following co-culture, TEER increased relative to pre-infection at all MOIs but maximum NTHi recoverability was at MOI 50 (median colony forming units, $6.8 \times 10^6$ per $cm^2$). SEM confirmed NTHi biofilm formation at MOI 50.

### Conclusions

A successful NTHi biofilm co-culture model on human primary epithelia was established. This will be used with PCD epithelia to investigate host-pathogen interactions during NTHi biofilm colonisation.

**P412**

# Understanding different outcomes of host-pathogen interactions of *L.monocytogenes* at the single cell level

Josephine Moran, Marie Goldrick, Elizabeth Lord, Ian Roberts, Pawel Paszek
University of Manchester, Manchester, United Kingdom

## Abstract

*Listeria monocytogenes* is a major foodborne pathogen with high mortality rates (40%). With a growing population of at-risk groups, including the elderly and immunocompromised, as well as an increase in use of processed foods, the major source of *L.monocytogenes* outbreaks, there is a greater need than ever to understand the host and pathogen factors that contribute to listeriosis.

Activation of pathogen virulence and antibacterial immune responses are inherently heterogenous, resulting in probabilistic outcomes of host-pathogen interactions at the single cell level. Here we use quantitative live-cell imaging approaches to understand interactions between *L.monocytogenes* and macrophages, a key niche for *L.monocytogenes* that promotes dissemination in the host.

We demonstrate there are different outcomes to *L.monocytogenes*-macrophage interactions; only a subset of *L.monocytogenes* establish a replicative niche within the host cell population, while remaining bacteria are killed or persist in a non-replicative state. Dual fluorescent reporter assays showed outcomes are not correlated within the same host cell, suggesting a bacterial factor controls the infection outcome. Despite heterogenous expression of *L.monocytogenes* PrfA regulated genes, this phenomenon is independent of PrfA activation. Furthermore, we found probability of replication depends on the multiplicity of infection (MOI). Higher MOI surprisingly reduces the probability of replication due to altered macrophage phagocytosis rate, emphasising the role of the host in infection outcome. This work highlights novel aspects of the role of host and pathogen in controlling infection outcome that could only be detected using single cell approaches. Further work will aim to identify the factors responsible for these phenomena.

**P413**

# Construction and Test Of An Efficient Biophotonic Imaging (BPI) Reporter System To Study Pneumococcal Biology In Vitro And In Vivo

Nada Alkhorayef
Life sciences, Leicester, United Kingdom

## Abstract

Streptococcus pneumoniae is a common nasopharyngeal resident in healthy persons, but it remains a major cause of pneumonia, bacteremia and otitis media despite vaccines and effective antibiotics. There is an urgent need for novel therapeutic approaches, but such advances require a detailed knowledge of S. pneumoniae biology. To better understand pneumococcal biology and infections, we need sensitive in vivo imaging technologies. To this end, bioluminescence imaging can be used, for example, to evaluate anti-infectives, intraspecies interactions and pneumococcal virulence non-invasively. Pneumococcal strains containing click beetle luciferase (CBRluc) under the control of putative highly expressed
pneumococcal promoters were constructed. TheCBRluc constructs were integrated into known sites in the S. pneumoniae genome. The bioluminescent strains were compared to a lux-based system expressing bacterial luciferase using in vitro growth experiments and in vivo mouse model of pneumonia. The results revealed that CBRluc tagged bacteria showed robust activity of bioluminescence inexponential phase that is also maintained during stationaryphase, whereas lux-expressing pneumococci emitted a light signal with high background that peaked during exponential phase and was significantly reduced in intensity during stationary phase. The presence of CBRluc did not affect the growth and virulence properties of the bioluminescent pneumococcal strains. In vivo bioluminescence activity obtained with CBRluc labelled bacteria was much higher than the lux containing strain under different promoters. This study also established that CBRluc reporter system can be used to study pneumococcal virulence in G. mellonella model.

The findings demonstrate that the CBRluc reporter system ismore efficient than lux, providing a potential platform for utilization in understanding of the mechanisms of pneumococcal pathogenesis in vivo.

**P414**

# Proline Tyrosine Kinase 2 inhibition alters survival and replication of adherent-invasive *Escherichia Coli* in macrophages

Xiang Li, Michael Ormsby, Lynsey Meikle, Damo Xu, Daniel Walker, Donal Wall
University of Glasgow, Glasgow, United Kingdom

## Abstract

Adherent-invasive *Escherichia coli* (AIEC) have been implicated in the aetiology of Crohn's Disease (CD). AIEC are characterised by an ability to adhere to and invade intestinal epithelial cells and replicate intracellularly in macrophages with macrophages from CD patients unable to control the replication of AIEC. However, little is known about how macrophage killing is impeded. We used an in vitro infection model to identify macrophage proteins associated with AIEC intracellular replication. Proline-rich tyrosine kinase 2 (PYK2) levels were identified as being significantly altered during AIEC infection. The PYK2 inhibitor PF-431396 significantly decreased intramacrophage replication of AIEC as determined by viable colony count, fluorescence immunostaining and imaging flow cytometry. PYK2 has previously been identified as a risk locus in inflammatory bowel diseases through genome wide association studies and is overexpressed in patients with intestinal and colorectal cancer (CRC), the latter a major long-term complication of CD. Future studies of the mechanism of PYK2 within infected macrophages will help to formulate a more cohesive mechanism how and where pathways interact and thus gain better insight into the dynamics of host–pathogen interactions.

## P415

# Removing the GAP: The effects on Rab32-mediated *Salmonella* killing

Kieran Sefton, Massimiliano Baldassarre, Stefania Spano
University of Aberdeen, Aberdeen, United Kingdom

## Abstract

The survival and replication of the bacterium *Salmonella enterica* serovar Typhimurium within macrophages relies upon delivery of the bacterial effectors GtgE and SopD2. These effectors target the GTPase Rab32 to prevent its recruitment to compartments known as *Salmonella* Containing Vacuoles (SCV). Rab32 activation and recruitment are critical steps for bacterial killing. Therefore, absence of these effectors in a *Salmonella* Typhimurium Δ*gtgE*Δ*sopD2* strain as well as in the human-adapted *Salmonella* Typhi, results in bacterial killing in murine macrophages. Modulation of the Rab32-dependent pathway will both increase our understanding of Rab32 trafficking and have important implications in combatting bacterial infection. Activation of Rab32 and its delivery to SCV occurs through exchange of GDP for GTP, whilst its GTPase activity is catalysed via interaction with GTPase-activating Proteins (GAPs). Here we show that the mRNA expression of certain GAPs is downregulated in macrophages in response to different infectious stimuli. We then investigated the effect of this expression modulation finding that some GAPs contribute significantly to both the localisation of Rab32 to SCV and to subsequent bacterial killing. We therefore propose Rab GAPs as negative regulators of a Rab32-dependent antimicrobial pathway and suggest that their inhibition would represent a novel, completely unexplored strategy to boost innate immunity and fight infection.

## P416

# K1 Escherichia coli form foci in the brain at 24h post infection

Zydrune Jasiunaite[1], Ryan Hames[1], Shiying Tang[1], Mariagrazia Pizza[2], Marco R. Oggioni[1]
[1]University of Leicester, Leicester, United Kingdom. [2]GSK Vaccines, Siena, Italy

## Abstract

K1 capsule type Escherichia coli is the predominant cause of neonatal meningitis. In an intravenous (IV) infection model with a clinical K1 isolate IHE 3034 adult mice die of sepsis within 24 hours. However, by utilising cefazolin, a first-generation cephalosporin known for its inability to pass through the blood brain barrier, it is possible to prevent bacteraemia and study the early phases of E. coli meningitis in the brain. Mice were infected IV with E. coli IHE 3034 and treated with cefazolin at 12 and 24h intraperitoneal to ensure the survival of animals to the determined end points. The results of the experiment show IHE 3034 is present in the brain as early as 12h and it remains in the tissue 72h post infection even if blood is clear. Despite this, at the cellular level, clear foci formation can be observed in the meninges and the choroid plexus around the vascular endothelial cells at 48h, showing IHE 3034's ability for intracellular replication inside the meningeal region The foci do not appear to be present at 24 or 72h, indicating for a secondary replication site around the major vascular endothelia sites of the brain. This further promotes the bacterial load increase through cell bursting in the tissue with or without a sustained bacterial influx from the blood. These findings show that K1 E. coli can form foci in the brain which may lead to escalated disease progression and severe morbidity following an initially successful clearance of bloodborne bacteria during sepsis.

**P417**

# Developing a novel *in vivo* burn wound model to study burn wound infection and to identify new wound therapeutic options.

Lara Eisaiankhongi[1], Evgenia Maslova[1], Yejiao Shi[2], Helena S Azevedo[2], David W Wareham[3], Folke Sjöberg[4,5], Ronan R McCarthy[1]

[1]Division of Biosciences, Centre for Inflammation Research and Translational Medicine, Department of Life Sciences, College of Health and Life Sciences, Brunel University London, London, United Kingdom. [2]Institute of Bioengineering, Queen Mary, University of London, London, United Kingdom. [3]Antimicrobial Research Group, Blizard Institute, Queen Mary, University of London, London, United Kingdom. [4]he Burn Centre, Department of Hand and Plastic Surgery, Linköping University, Linköping, Sweden. [5]Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden

## Abstract

Despite significant improvements in wound care and infection control in the 21st century, wound infection is still one of the main clinical complications associated with wound management. In burn patients in particular, the leading cause of mortality is infection. Burn wound infection can also lead to autograft failure and prolonged healing regimes, placing a huge financial burden on global health care systems. The challenge of treating and preventing wound infections has been exacerbated by the emergence of antibiotic resistance. This has led to outbreaks of multidrug resistant pathogens in burn centres around the world. Animal burn wound models to study pathogenicity and to develop new therapeutic options are governed by strict ethical guidelines. They are also hindered by high levels of animal suffering and the extensive training that is required to achieve consistent reproducible results. This has led to a therapeutic discovery bottle neck. Here, we describe a less ethically challenging invertebrate model of burn trauma and concomitant wound infection. We demonstrate that this model recapitulates many of the hallmarks of burn trauma and wound infection seen in mammalian models and in human patients. We also outline how this model can be used to discriminate between high and low pathogenicity strains of some of the most common burn wound colonisers *Pseudomonas aeruginosa* and *Staphylococcus aureus,* and *Acinetobacter baumannii.* We also describe how we have used this model to identify specific skin commensal bacteria that when applied to a burn wound can limit infection progression.

**P418**

# Sequence variation and role of FdeC in Avian Pathogenic *Escherichia coli* adhesion

Adrianna Aleksandrowicz, Rafał Kolenda
Wrocław University of Environmental and Life Sciences, Department of Biochemistry and Molecular Biology, Wrocław, Poland

## Abstract

FdeC is intimin-like adhesin present in genomes of pathogenic *E. coli,* however *fdeC* gene is disrupted in several non-pathogenic strains. Our preliminary results of 2000 transposon mutants from APEC IMT5155 strain revealed increased adhesion ability of the strain with transposition in the coding sequence of *fdeC* to chicken intestinal cells. Therefore we aimed to determine the role of the *fdeC* gene in APEC adhesion. For this purpose, we analyzed the prevalence and sequence variation of FdeC using 570 APEC and 2200 non-pathogenic *E. coli* genomes. Next, we investigated biochemical conditions of *fdeC* expression and based on this knowledge scrutinized the role of *fdeC* as a virulence factor in experiments fulfilling molecular Koch's postulates. FdeC was absent in only 6.6% of APEC and more than 25% of non-pathogenic strains. Furthermore, we detected that 9 out of 627 FdeC variants were specific to 40% of APEC strains and differed in primary structure by 6 to 8% from most prevalent variants in non-pathogenic *E. coli.* Subsequently, we established that *fdeC* expression is regulated by temperature, pH, and sodium acetate concentration. Adhesion assays in FdeC expression-inducing conditions demonstrated 20% higher adhesion ability of *fdeC* deletion mutant in comparison to its wild type strain. Moreover, the complementation of mutation revealed this adhesive phenotype is altered solely due to *fdeC* deletion. To summarize, we have shown the presence of APEC-specific FdeC variants and suggested FdeC deletion is compensated by overexpression of other virulence factors contributing to *E. coli* adhesion.

**P419**

# Prevalence of *Cryptosporidium parvum* in dairy cows farms from the Netherlands, Belgium and France

Pedro Pinto[1], Claudia A. Ribeiro[1], Sumaiya Hoque[1], Gary Robinson[1], Evi Canniere[2], Yvonne Daandels[3], Martine Dellevoet[3], Janine Roemen[3], Anne Bourgeois[4], Ourida Hammouma[5], Jérôme Follet[5], Martin Kváč[6], Anastasios D. Tsaousis[1]
[1]University of Kent, Canterbury, United Kingdom. [2]Inagro, Rumbeke, Belgium. [3]ZLTO, Den Bosch, Netherlands. [4]Selas CVE, Dainville, France. [5]YNCREA-ISA, Lille, France. [6]Institute of Parasitology, Biology Centre CAS, České Budějovice, Czech Republic

## Abstract

*Cryptosporidium* genus is comprised of protozoan parasites, which infect a wide range of hosts, causing a disease called cryptosporidiosis. In cattle-farms, the incidence of cryptosporidiosis results in high mortality and, consequently, is a source of considerable economic loss in the livestock industry. Infected animals also might act as major reservoir of *Cryptosporidium* spp., in particularly *C. parvum*, the most common cause of cryptosporidiosis in cattle, and thus pose a significant risk to other farms via breeding centers, to the trading of livestock, and to human health. This study, funded by the Interreg-2-seas, aims to assess *C. parvum* prevalence across dairy farms in the Netherlands, Belgium, and France, and further investigate the zoonotic potential of the circulating *C. parvum* subtypes. To accomplish this, 1084 cow stool samples, corresponding to 57 dairy-farms from all three countries, were analysed. Well-established protocols amplifying the *18S*-rRNA and *gp60* genes fragments, followed by DNA sequencing, were used for the detection and subtyping *C. parvum*; the DNA sequences obtained were further characterised using a combination of bioinformatics and phylogenetics methods. Our results show 18.05%, 17.01% and 15.14% prevalence of *C. parvum* in the Netherlands, Belgium, and France respectively. The *gp60* subtyping carried out demonstrated a significant number of the *C. parvum*-positives belongs to the IIa allelic family, which has been also detected in humans. Consequently, this study highlights how widespread is *C. parvum* in dairy-farms and endorses cattle as a major carrier of zoonotic *C. parvum* subtypes, which subsequently pose a significant threat to human health.

## P420

# Mechanistic insight into the role of the membrane protein MspA for *Staphylococcus aureus* virulence

Dora Bonini, Seána Duggan, Tarcisio Brignoli, Ruth Massey
University of Bristol, Bristol, United Kingdom

## Abstract

*Staphylococcus aureus* is a major human pathogen and increasingly a threat to global health, due to the circulation of antibiotic resistant strains. It can colonise the host asymptomatically but also cause invasive disease via its arsenal of secreted toxins and immune evasion factors. A genome-wide association study has linked a previously uncharacterized protein, which we have named membrane stabilising protein A (MspA), to *S. aureus* toxicity. Inactivation of MspA causes downregulation of the main virulence regulator, the Agr system, decrease in secretion of phenol-soluble modulins (PSMs) toxins, higher sensitivity to different immune cells and increased intracellular iron concentrations. Despite affecting a range of phenotypes, it is still unclear how MspA operates at a molecular level. To characterise MspA localisation and identify interacting partners, the *mspA* gene was fused with the mcherry fluorescent tag. The fusion was cloned into a plasmid and transformed into the *mspA* mutant. The fusion protein does not rescue toxicity to wild-type levels. However, SDS-PAGE of whole cell lysates show intracellular levels of PSMs comparable to the wild-type. This suggests partial complementation of toxicity and restoration of the Agr system functionality. Western blots and preliminary fluorescence microscopy data indicate that the MspA-Mcherry fusion localises exclusively in the membrane fraction. Co-immunoprecipitation with RFP-Trap® and proteomics analyses are currently underway to identify proteins potentially interacting with MspA. A bacterial two-hybrid system will be then used to verify protein interactions. This experimental strategy will highlight MspA partners and help elucidating MspA role in *S. aureus* virulence.

## P421

# The Roles of Outer Membrane Vesicles of *Helicobacter pylori* in Pathogenesis

Lolwah Alsharaf[1,2], Lesley Hoyles[1], David J Boocock[3], Jody Winter[1]
[1]Department of Biosciences, Nottingham Trent University, Nottingham, United Kingdom. [2]Al-Amiri Hospital, Ministry of Health, Kuwait City, Kuwait. [3]John van Geest Cancer Research Centre, Nottingham Trent University, Nottingham, United Kingdom

## Abstract

**Background:** *H. pylori* constantly releases outer membrane vesicles (OMV) during growth. These vesicles contain virulence factors and are cytotoxic, but the properties and contents of bacterial OMV can vary depending on the environmental conditions.

**Project aim:** To characterise the protein contents and cytotoxicity of OMV isolated from *H. pylori* grown under different environmental conditions.

**Methods:** OMV were purified from *H. pylori* cultures in brain heart infusion broth with 0.2% β-cyclodextrin after 1 or 6 days, or blood agar plates after 24 hours. OMV toxicity on human gastric epithelial (AGS) cells was determined by CellTiter assay and IncuCyte. Proteins were characterised by LC-MSMS and quantified, label-free, by SWATH-MS to determine changes in protein expression.

**Results:** *H. pylori* OMV isolated from late broth and plate cultures were significantly more toxic to AGS cells than those isolated from early broth ($p < 0.01$). Vacuoles were seen in AGS cells treated with OMV isolated from early broth and plate cultures, indicating activity of the vacuolating cytotoxin VacA. There was ~2-fold more VacA protein in OMV from early broth and plate cultures than those from late broth. In contrast, there was more of the other major toxin CagA in OMV from early broth than in OMV from late broth and plate cultures. OMV isolated from plate cultures had completely different protein profiles compared with OMV from broth cultures.

**Conclusion:** Characteristics of *H. pylori* OMV change dramatically, depending on the environment in which the bacterium is grown. This may influence bacterial virulence.

**P422**

# From the lab to the clinic: transcriptome analysis of *Pseudomonas aeruginosa* biofilm infection in an *ex vivo* pig lung model of the cystic fibrosis lung

Niamh Harrington, Jenny Littler, Freya Harrison
School of Life Sciences, University of Warwick, Coventry, United Kingdom

## Abstract

Chronic *Pseudomonas aeruginosa* biofilm infections in the cystic fibrosis (CF) lung are associated with high levels of antimicrobial resistance and increased mortality rates. We are using a high-throughput, clinically realistic *ex vivo* pig lung (EVPL) model to mimic *P. aeruginosa* biofilm infections in CF. We performed RNA sequencing of *P. aeruginosa* biofilms growing on porcine bronchiolar tissue, cultured with synthetic mucus media over a period of seven days, to better understand the dynamics of colonisation and acclimation to the CF lung environment. RNA sequencing was also performed on *P. aeruginosa* growing in synthetic mucus media in the absence of bronchiolar tissue. We demonstrate a clear difference in *P. aeruginosa* gene expression between *in vitro* and EVPL model growth, and the creation of different niches within the EVPL model. These differences have functional importance for phenotypes linked to virulence and antibiotic tolerance. Multiple quorum sensing pathways were found to be downregulated in the EVPL model, compared with *in vitro*. This is consistent with published results comparing expression during human infection with laboratory studies. Our data indicates that the EVPL grown *P. aeruginosa* transcriptome is closer to that seen in human infection than other current lab models have been able to achieve. Hence, our model shows the potential to bridge the gap between the laboratory and true CF lung infection and highlights key infection dynamics that may play a role in the persistence and severity of chronic *P. aeruginosa* biofilm infections in CF.

## P423

# Sessile *Clostridioides difficile* contribute towards recurrent *C. difficile* infection

Charmaine Normington[1], Ines Moura[1], Jessica Byrant[2], Duncan Ewin[1], Christopher Ford[2], Mark Wilcox[1], Anthony Buckley[1]
[1]University of Leeds, Leeds, United Kingdom. [2]Seres Therapeutics, Massachusetts, USA

## Abstract

*C. difficile*, an anaerobic spore-forming intestinal pathogen, produces up to three toxins that cause host cell damage resulting in disease, *C. difficile* infection (CDI). Therapies include antibiotic treatment; however, up to 30% of cases fail primary therapy, resulting in recurrent disease, which increases patient morbidity and places a burden on worldwide healthcare systems. We have little understanding of why these therapies fail. Using a clinically validated *in vitro* gut model, we assess the contribution of biofilms towards recurrent disease and to investigate biofilm microbiota-*C. difficile* interactions. During induction of simulated CDI, *C. difficile* spores and vegetative cells became associated with the colonic biofilm microbiota. Vancomycin treatment did not effectively remove the biofilm *C. difficile* cells and recurrent infection was observed. Additionally, vancomycin therapy followed by faecal microbiota transplant did resolve the recurrent infection, but the biofilm *C. difficile* cells remained unaffected. In a biofilm transfer experiment, we showed that transferring biofilm encased *C. difficile* cells into a *C. difficile* naïve (but CDI susceptible model) induced CDI. Furthermore, we show that members of the biofilm community can impact *C. difficile* biofilm formation either acting in an antagonistic or synergistic manner. We highlight the importance of biofilms as a reservoir for *C. difficile*, which can be a cause for recurrent infections.

**P424**

# The Rab32/BLOC-3 dependent pathway mediates host-defence against different pathogens in human macrophages.

Massimiliano Baldassarre[1], Virtu Solano-Collado[1], Arda Balci[1], Rosa A Colamarino[1], Ivy M Danbuza[2], Heather M Wilson[1], Gordon Brown[2], Subhankar Mukhopadhyay[3], Gordon Dougan[4], Stefania Spanò[1]
[1]Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom. [2]MRC Centre for Medical Mycology, University of Exeter, Exter, United Kingdom. [3]MRC Centre for Transplantation, Peter Gorer Department of Immunobiology, School of Immunology and Microbial Sciences, King's College London, London, United Kingdom. [4]MRC Centre for Medical Mycology, University of Exeter, Cambridge, United Kingdom

## Abstract

Host defence mechanisms protect complex organisms against the attack of microbes. To elucidate these mechanisms, we investigate the strategies that intracellular pathogens have evolved to counteract macrophage killing and establish infections. Macrophages provide a first line of defence against microorganisms, and while some mechanisms to kill pathogens such as the oxidative burst are well described, others are still undefined or unknown. The Rab32 GTPase and its guanine nucleotide exchange factor BLOC-3 are central components of a trafficking pathway that controls intracellular *Salmonella* replication delivering itaconic acid to the Salmonella containing vacuole. Here we show that this pathway is active both against bacterial and fungal intracellular pathogens. We also show that it controls *Salmonella* Typhi replication in human macrophages and that, to survive in them, *Salmonella* Typhi actively counteracts the pathway through its *Salmonella* pathogenicity island-1-encoded type III secretion system. These findings demonstrate that the Rab32/BLOC-3 pathway is a novel and universal host-defence pathway and protects mammalian species from a wide range of intracellular pathogens.

**P425**

# Genital Tract Gammaherpesvirus Reactivation is Linked to Female Reproductive Loss in European Badgers (Meles meles)

Ming-shan Tsai[1], Ursula Fogarty[2], Andrew W. Byrne[3], James O'Keeffe[3,4], David Macdonald[1], Christina Buesching[5]
[1]University of Oxford, Oxford, United Kingdom. [2]Irish Equine Centre, Naas, Ireland. [3]Agriculture House, Dublin, Ireland. [4]University College Dublin, Dublin, Ireland. [5]University of British Columbia, Vancouver, Canada

## Abstract

Reactivation of latent Gammaherpesvirus in the genital tract can lead to reproductive failure in domestic animals. Nevertheless, this pathophysiology has not received formal study in wild mammals. High prevalence of Mustelid gammaherpesvirus 1 (MusGHV-1) DNA detected in the genital tracts of European badgers (Meles meles) implies that this common pathogen may be a sexual transmittable infection, which may cause certain level of sterility. Here we used PCR to test MusGHV-1 DNA prevalence in genital swabs collected from 144 wild badgers in Ireland (71 males, 73 females) to investigate impacts on male fertility indicators (relative sperm abundance in epididymis and testes weight) and female fecundity (current reproductive outcome). MusGHV-1 reactivation is a risk factor on failed female reproduction (Chi-squared test, odds ratio = 0.269, p-value = 0.017), but not on male fertility; however males had a higher risk of MusGHV-1 reactivation than females (Chi-squared test, odds ratio=3.08, *p*-value=0.003), especially during the late-winter mating season. Negative results in foetal tissues from MusGHV-1 positive mothers indicated that cross-placental transmission was unlikely. This study has broader implications for how wide-spread and asymptomatic gammaherpesvirus infections could affect reproductive performance in wild Carnivora species. Further studies on the underlying mechanism of MusGHV-1 related abortion should be conducted, especially from the aspects of local microbiome compositions and immune activities.

## P426

# Wall teichoic acid: holding the balance between toxicity and fitness

Tarcisio Brignoli[1], Edward Douglas[1], Olayemi Grace Fagunloye[2], Rajan Adhikari[3], M. Javad Aman[3], Ruth Massey[1]
[1]School of Cellular and Molecular Medicine, University of Bristol, Bristol, United Kingdom. [2]School of Medicine, University of Pittsburgh, Pittsburgh, USA. [3]Integrated Biotherapeutics, Inc. (IBT), Rockville, USA

## Abstract

*Staphylococcus aureus* is a major human pathogen, able to express a plethora of virulence factors, including several cytolytic toxins. A genome wide association study carried out on a collection of ST239 clinical isolates identified a single nucleotide polymorphism (SNP) associated with an increase in the cytolytic capacity (toxicity) of the staphylococcal strains. The SNP is in the intergenic region between the *tarK* and *tarF* genes, which are part of the wall teichoic acid (WTA) biosynthetic pathway. WTAs are important polymers bound to the peptidoglycan which are involved in several cell functions, ranging from cell division to host interactions. The new allele results in higher expression of the downstream gene (*tarF*), and the isolates having this variant produce more WTA than the isolates with the most common allele. Using a molecular genetic approach, we show that lower WTA production is associated with lower toxin secretion. On the other hand, strains that produce less WTA can grow to higher cell densities, as they are able to switch nutrient sources to avoid limitation of a primary nutrient consumption. We hypothesise that an imbalance in WTA intermediates determines changes in the activity of the PhoPR two component system, which has an impact on *S. aureus* metabolism. The emergence of different alleles controlling *tarF* expression seems to reflect different strategies across *S. aureus* clinical isolates, with a trade-off between toxicity and the ability to grow in a low nutrient environment

**P427**

# Defining the pknH Transcriptional Network in the Animal Tuberculosis bacillus, *Mycobacterium bovis*

Anne Kerins, Damien Farrell, Stephen V. Gordon
University College Dublin, Dublin, Ireland

## Abstract

The *Mycobacterium tuberculosis* complex (MTBC) is a group of bacteria that show more than 99% genetic identity, yet they diverge in their host preference and the severity of associated disease. *Mycobacterium bovis* causes the disease in cattle and poses an economic challenge, with the cost of Irelands TB Eradication Scheme predicted to exceed €90 million in 2020.

The aim was to focus on the RD900 and *pknH* gene network in MTBC members. The *pknH* gene encodes a transmembrane serine threonine protein kinase, that plays a role in the regulation of signalling pathways in the mycobacterial cell and has been linked to virulence. The RD900 or "region of difference 900" contains the *tbD2* and *pknH2* genes. The part of the genome that contains this region varies substantially across MTBC members.

The results of RNA-seq analysis showed that there are statistically significant levels of differential gene expression in wildtype and knock-in *M. bovis* strains.

The RD900 analysis across the MTBC showed that there is variation in this important genomic region. Analysis carried out by Mata et al. 2020, showed that this RD900 region had been independently lost in different MTBC lineages and strains. *M. bovis* has retained the RD900 region and this is believed to be linked to its increased virulence.

The majority of the *M . africanum* strains were found to possess the standard *pknH, tbD2, pknH2*, *embR* gene arrangement as is found in *M. africanum* GM041182, the strain that is typically used as a reference.

Based on work of Mata et al., 2020.

## P428

# Identification of a novel putative transcription factor in *Chlamydia trachomatis.*

Srishti Baid, Michael L Barta, Scott Lovell, P Scott Hefty
University of Kansas, Lawrence, USA

## Abstract

*Chlamydia trachomatis* is a worldwide public health challenge as the primary cause of bacterial sexually transmitted infections and blindness. These are obligate intracellular bacteria that are maintained through a biphasic developmental cycle that includes conversions between distinct infectious elementary bodies and non-infectious, replicative reticulate bodies. The ability of the organism to infect and cause disease is dependent on careful regulation of conversion and replication processes. The regulatory factors and mechanisms that control the conversion processes of the developmental cycle are still being discovered and characterized. Through protein structure determination and database analysis, a protein of unknown function (CT457) is hypothesized to serve as a transcription factor and contribute to the regulation of chlamydial processes. Protein structure similarity supports functional annotation as a YebC/PmpR transcription factor which homologs have been shown to control a diverse array of genes in many bacteria. To investigate the potential function of CT457 as a transcription factor and identify an associated regulon, RNA seq experiments were performed in *Chlamydia* following expression induction. Additionally, morphologic analyses were performed following overexpression to potentially discover contributions to growth or conversion processes. Further studies will be performed to evaluate the role of CT457 and decipher its importance in chlamydial biology.

## P429

# Quorum sensing dependent cooperation and conflict in bacterial aggregates

Kathleen O'Connor, Stephen P. Diggle
Center for Microbial Dynamics and Infection, School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA

## Abstract

*Pseudomonas aeruginosa* is an opportunistic pathogen commonly found in cystic fibrosis (CF) lungs, where it mutates and diversifies. *P. aeruginosa* quorum sensing (QS) mutants are commonly isolated from CF lungs and are an indicator of worsening chronic infections. Given that QS is a cooperative behavior, facilitating *P. aeruginosa* to colonize new hosts and cause infection, it is still unknown why QS mutants are so frequently found in infections. During infection, *P. aeruginosa* grows in aggregates of 10-10,000 cells but to date, little is known about how social behaviors such as QS function are maintained in aggregates. To understand this more fully, we used two environments where QS mutants may either gain or provide fitness benefits to other cells. In one environment, QS mutants 'cheat' on wild-type cells, and in the other, QS mutants may benefit the whole population by conferring resistance to beta-lactam antibiotics. Our work is preliminary and we will report on our findings. The work should shed further light on the role of microbial dynamics on the fitness of *P. aeruginosa* populations in bacterial aggregates.

## P430

# Sequestosome-1: A novel Rab32 interacting protein

Arda Balci, Massimiliano Baldassarre, Stefania Spanò
University of Aberdeen, Aberdeen, United Kingdom

### Abstract

The small Rab GTPases are a master regulator of vacuole trafficking and phagosome maturation, thereby manipulated by various intracellular pathogens. Indeed, *Salmonella* Typhimurium secreted two effector proteins GtgE and SopD2 allow the bacterium to establish infection in macrophages through cleaving and deactivating Rab32. Intriguingly, acquisition of Rab32 to *Salmonella* Typhi-containing vacuoles results in clearance of the pathogen in macrophages. However, the Rab32-mediated killing mechanism is not well understood. Here, we wanted to identify Rab32 interacting proteins in mouse macrophages infected with *S.* Typhimurium Δ*GtgE*Δ*SopD2*, a strain that lacks GtgE and SopD2 proteins using immunoprecipitation coupled with LC-MS/MS. Proteomics analysis revealed Sequestosome-1 (p62) as a candidate Rab32 interacting protein. We confirmed the interaction using co-immunoprecipitation assay and demonstrated the recruitment of GFP-p62 to the *Salmonella*-containing vacuoles in macrophages. Furthermore, we assessed the survival of *S.* Typhimurium Δ*GtgE*Δ*SopD2* in p62 knock-down macrophages and showed a 4.7-fold increase in bacterial burden in p62 depleted cells. Notably, we revealed that *Salmonella* Typhi, which is a human-restricted pathogen that cannot establish infection in mice partially due to Rab32-mediated killing, survives significantly better (2.2-fold) in p62 deficient murine macrophages. These results suggest that in addition to Rab32, p62 also contribute to *Salmonella* Typhi host restriction. Finally, using transmission electron microscopy we showed that in p62 knock-down macrophages *Salmonella* resides in a single-membrane vacuole at 24 hours post-infection, suggesting that the observed phenotype is not due to a cytosolic hyper-replication. Further investigation is required to establish the importance of Rab32 and p62 interaction in macrophages.

**P431**

# Immunoproteomic analysis of surface proteins in bovine-adapted lineages of *Staphylococcus aureus*.

Shauna. D Drumm[1,2], Paul Cormican[1], Rebecca Owens[3], Jennifer Mitchell[2], Orla M. Keane[1]
[1]Animal and Bioscience Research Department, Teagasc, Grange, Co. Meath, Ireland. [2]School of Biomolecular and Biomedical Science, University College Dublin, Belfield, Dublin 4, Ireland. [3]Department of Biology, Maynooth University, Maynooth, Co. Kildare, Ireland

## Abstract

In Ireland, *Staphylococcus aureus* is the most frequent cause of bovine intramammary infection (IMI) with the bovine-adapted lineages CC151 and CC97 most commonly found. While bovine mastitis vaccines are available that target *S. aureus*, this pathogen is still a significant source of infection in dairy cows. Therefore, there's a clear need for a more effective vaccine against *S. aureus*. However, *S. aureus* is considered a clonal organism, therefore identifying new potential protein targets common to all major lineages is an important step for vaccine design.

Two *S. aureus* strains, one belonging to ST151 and another to ST3170 (CC97), were used to infect two groups of dairy cows. Antibodies raised by individual cows were used to identify immunodominant surface proteins for each strain. One-dimensional serum blotting determined that the antibody response to *S. aureus* infection was largely strain specific, and to a lesser extent, animal specific.

Two- dimensional serum blotting followed by mass spectrometry of immunoreactive spots was used to identify potential vaccine candidates that were immunodominant for both strains. These blots showed that proteins to which an antibody response was generated in the ST151 infected cows were generally different to those generated in the ST3170 infected cows. However, mass spectrometry also identified immunoreactive proteins common to both strains including Clumping factor B and Iron-regulated surface determinant protein A. Common immunoreactive secreted proteins are also currently being identified.

This study will identify immunodominant proteins expressed by the bovine-adapted strains ST3170 and ST151 that could potentially be used as candidates in vaccine research.

## P432

# Investigation of the influence of plant tissue damage on the attachment of Listeria to fresh salad produce

Asma Alsharif
univeristy of leicester, leicester, United Kingdom

## Abstract

Vegetables and fruits are an important for a healthy life due to them containing nutritionally important minerals and vitamins. Green salads (spinach, lettuce) are popular convenience consumer products, however evidence is increasing that these ready to eat salads have been identified as a source of foodborne illness. There are many pathogenic bacteria which are transmitted by food that can cause serious disease. One of the most important is *Listeria monocytogenes* because it can cause invasive infections and especially because of its ability to grow at low (food refrigeration) temperatures. The aim of this study is to investigate whether compounds released from damaged fresh salad leave have an effect on *Listeria* growth and virulence. The results in this report show that *Listeria* was highly responsive to the salad extracts and that growth and biofilm formation were both increased compared to un-supplemented control cultures. Plant derived chemicals also stimulated *Listeria* growth in serum-SAPI medium. The results also showed that *Listeria* treated with salad extract was more virulent in a *Galleria mellonella* infection model.

## P433

# ‘I’m like a fungus; you can’t get rid of me’ or can you? An Audit of Candidaemia Management Over One Year in an Irish Tertiary Care University Hospital

Luke O'Brien, Saied Ali, Eoin Conlon, Neil Wrigley Kelly, Sinead McNicholas, Sarmad Waqas
St Vincent's University Hospital, Dublin, Ireland

## Abstract

**Background**: Candidaemia is the most common manifestation of invasive candidiasis. Considering its associated morbidity and mortality, an audit of candidemia at our institute was undertaken.

**Aim**: To assess compliance with the local and international candidemia management guidelines and to ascertain its clinical outcomes.

**Methods**: A retrospective medical record review was conducted for all patients diagnosed with candidaemia from September 2019 to September 2020 inclusive following approval from the hospital clinical audit department in accordance with GDPR.

**Results:** A total of 17 patients were diagnosed with candidemia over the study period, only 15 patients were included in the analysis as two patients died *before* *Candida* was isolated from their blood cultures. Infection was primarily hospital-acquired (80%, n=12). Source of infection varied from intra-abdominal (46.7%, n=7), to intravenous (IV) line associated (40%, n=6) and genitourinary (13.3%, n=2). Early IV treatment with an echinocandin or an acceptable alternative was initiated for all patients with all receiving appropriate definitive antifungals. For patients with IV-line-associated candidiasis, the line was removed in all cases; and for those with an intra-abdominal source, 57.1% (n=4) had appropriate interventions to achieve source control. Follow-up cultures were repeated in 93.3% (n=14) appropriately. An echocardiogram and ophthalmological exam were performed for 86.6% of patients (n=13). Treatment was continued for minimum of two weeks after negative cultures in 80% (n=12). Two patients unfortunately died during their course of treatment due to consequences of their underlying disease process, while all active management was withdrawn in the other. Mortality within 1-month of diagnosis was 26.7% (n=4) and within 1-year was 46.7% (n=7).

**Conclusion:** Overall, guideline compliance was optimal with appropriate treatment and interventions. Failure to attain 100% is directly related to the morbidity and mortality of this patient cohort as reflected by the high mortality rates. Interventions to promote effective management of this important infection will be implemented and re-audited.

## P434

# Functional responses of neutrophils to *Fusobacterium nucleatum* subspecies

Maria Muchova, Sarah A. Kuehne, Melissa Grant, Iain Chapple, Josefine Hirschfeld
University of Birmingham, Birmingham, United Kingdom

## Abstract

### Introduction

*Fusobacterium nucleatum* is an opportunistic oral pathogen with five subspecies: *animalis, fusiforme, nucleatum, polymorphum* and *vincentii.* These play an important role in biofilms associated with chronic gum disease (periodontitis). Neutrophils form the first line of defence against these and other oral pathogens, employing a multitude of anti-microbial strategies, which, alongside bacterial killing, also cause collateral host-tissue damage. The immunogenic properties of *F. nucleatum* subspecies are poorly understood. The purpose of this study was to investigate specific neutrophil responses to all *F. nucleatum* subspecies.

### Methods

All *F. nucleatum* subspecies were grown planktonically overnight and inactivated using 10% neutral buffered formalin. Peripheral blood neutrophils (PBN) and neutrophil-like cells (differentiated from HL-60 cells) were challenged with inactivated bacteria (MOI 100) and production of reactive oxygen species (ROS) was quantified by Luminol/Iso-luminol enhanced chemiluminescence assays. Release of neutrophil extracellular traps (NETs) was quantified fluorescently using Sytox-green. Additionally, phagocytosis of fluorescently labelled *F. nucleatum* subspecies was analysed in both cell groups by flow cytometry.

### Results

In addition to differences in ROS generation by PBN and neutrophil-like cells in response to individual *F. nucleatum* subspecies, variations in the amount of extracellular DNA extruded during NETosis was evident. Moreover, distinct levels of phagocytic activity of both cell types will be shown.

### Conclusion

*F. nucleatum* pathogenicity has been reported in oral as well as systemic diseases (colorectal cancer, cardiovascular disease). Understanding subspecies-specific effects of *F. nucleatum* on neutrophils will enhance our knowledge of the interactions between this bacterium and the host immune response and may help to identify new therapeutic targets.

**P436**

# Microbiota-derived metabolites inhibit *Salmonella* virulent subpopulation development by acting on single-cell behaviors

Alyson Hockenberry[1], Gabriele Micali[2], Gabriella Takacs[1], Jessica Weng[3], Martin Ackermann[1]
[1]ETH Zurich, Zurich, Switzerland. [2]Eawag, Dübendorf, Switzerland. [3]Mayo Clinic, Rochester, USA

## Abstract

*Salmonella spp.* express *Salmonella* pathogenicity island 1 (SPI-1) genes to mediate the initial phase of interaction with host cells. Prior studies indicate short-chain fatty acids, microbial metabolites at high concentrations in the gastrointestinal tract, limit SPI-1 gene expression. A number of reports show only a subset of *Salmonella* cells in a population express these genes, suggesting short-chain fatty acids could decrease SPI-1 population-level expression by acting on per-cell expression and/or the proportion of expressing cells. Here, we combine single-cell, theoretical, and molecular approaches to address the effect of short-chain fatty acids on SPI-1 expression. Our results show short-chain fatty acids do not repress SPI-1 expression by individual cells. Rather, these compounds act to selectively slow the growth of SPI-1 expressing cells, ultimately decreasing their frequency in the population. Further experiments indicate slowed growth arises from short-chain fatty acid-mediated depletion of the proton motive force. By influencing the SPI-1 cell-type proportions, our findings imply gut microbial metabolites act on cooperation between the two cell-types and ultimately influence *Salmonella*'s capacity to establish within a host.

**P437**

# Aggregation and cell-cycle mediated invasion in *Listeria monocytogenes* infection

Liam Feltham, Pawel Paszek, Ian Roberts
University of Manchester, Manchester, United Kingdom

## Abstract

**Background**: *Listeria monocytogenes* is a food-borne pathogen, which needs to establish replicative niche in order to disseminate in the host. Most studies of infection have been performed at the population level, however at the single cell level host-pathogen interactions are known to be heterogenous and stochastic.

**Methods**: In order to understand host-pathogen interaction of the *L. monocytogenes* weemployed live-cell imaging approaches. We used multiparameter fluorescent protein reporters as markers for bacterial virulence gene expression upon infection of human cells. After 2 hours, the infection was treated with an antibiotic to kill the extracellular *L. monocytogenes.* Live cell imaging continued for 18 hours post-infection and the images were analysed at the single cell level.

**Results**: Although many bacteria were able to adhere to the membrane of the host cells, only a very small fraction of the total bacterial cell population were able to successfully invade the host cells. Successful invasion of host cells is linked to bacterial aggregates forming on the host cell surface, these aggregated bacteria are able to invade host cells simultaneously and increase the invasion success rate. These aggregates are induced by a secreted host cell factor resulting in increased PrfA virulence expression. There was also a striking correlation with the host cell cycle, the success of invasion was dependent on the phase of the host cell cycle.

**Conclusions:** Successful invasion is a rare event. Cell cycle gating and aggregation may represent strategies that allow to increase this invasion rate. Single cell approaches provide a new avenue to study the infection process.

**P438**

# How does *Aspergillus fumigatus* colonisation affect the progression of cystic fibrosis lung disease?

Zara Kaplan[1], Ormela Azate[1], Gordon Ramage[2], Freya Harrison[1]
[1]University of Warwick, Coventry, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom

## Abstract

*Aspergillus fumigatus* (*A. fumigatus*) is one of the most common fungi found in the airways of people with cystic fibrosis (CF). Allergic bronchopulmonary aspergillosis (ABPA), which can be triggered by inhaling *A. fumigatus* spores, has been associated with a decline in lung function. However, there is uncertainty over the effect of *A. fumigatus* colonisation without ABPA symptoms on the respiratory health of people with CF patients. A meta-analysis of published studies was performed to assess the effect of *A. fumigatus* colonisation on lung function in people with CF. High heterogeneity in the effect of *A. fumigatus* colonisation was found between studies. Hence, while we can draw a preliminary conclusion that *A. fumigatus* plays some role in CF lung disease, our work highlights the need for further, more focused research in this area to facilitate a clearer picture of its clinical significance.

**P439**

# Haem scavenging by pathogenic *Neisseriaceae* bacteria through haemoglobin receptor HpuAB

Daniel Akinbosede, Stephen Hare
University of Sussex, Brighton, United Kingdom

## Abstract

*Neisseria meningitidis* and *Neisseria gonorrhoeae* are two important human pathogens. *N. meningitidis* is responsible for bacterial meningitis and a death toll of tens of thousands globally per annum. *N. gonorrhoeae* is responsible for the sexually transmitted infection, gonorrhoea, classed by the WHO as a 'HIGH' priority bacterial pathogen, where new therapies are urgently needed in an effort to fight antibiotic resistance. Key to developing new therapies is understanding the ways in which the pathogen survives inside the host. Iron acquisition is one of the main barriers bacterial pathogens face and, to ensure survival, many pathogens have evolved creative systems that allow them to steal iron from iron-carrying host proteins. *Neisseria* are able to utilise haemoglobin (Hb) and the haemoglobin-haptoglobin (Hb:Hp) complex as an additional iron source through the haemoglobin-binding TonB-dependent receptor system HpuAB. The specifics of HpuAB functionality on a structural level is poorly understood, although significant progress was made by *Wong et al.* (2015) who described the structure and binding capabilities of HpuA, an extracellular lipoprotein that works in partnership with HpuB to facilitate the iron acquisition. There is currently no published structure of HpuB, however homology studies predict the protein to be a 22-strand amphiphilic β-barrel pore structure with an N-terminal plug domain. Here, we will describe our progress towards a structural and functional understanding of the role of HpuB in iron acquisition using biochemistry, biophysics, X-ray crystallography and electron microscopy (TEM).

## P440

# The role of air pollution and bacteria in COPD.

Lillie Faye Purser, Joanne Purves, Louise Corscadden, Daniella Lucy Spencer, Paul S Monks, Julian M Ketley, Peter W Andrew, Michael Barer, Christopher Brightling, Julie A Morrissey
University of Leicester, Leicester, United Kingdom

### Abstract

Air pollution is the single largest environmental health risk worldwide. Particulate matter (PM) air pollution is released as a result of fossil fuel combustion and vehicle motion, breaking and tyre wear. It has been shown that exposure to PM can cause increased levels of respiratory disease, including the exacerbation of COPD, which is frequently associated with bacterial infection. Despite this, the effects of air pollution exposure on COPD associated respiratory bacteria, such as *Haemophilus influenzae, Moraxella catarrhalis* and *Streptococcus pneumoniae* are largely unknown. Our recent publication was the first to document that as well as damaging the host, PM has a direct impact on bacteria that can cause respiratory infections. We showed that exposure to black carbon (BC), an important component of PM air pollution, results in alterations in biofilm structure in both *Streptococcus pneumoniae* and *Staphylococcus aureus*, and increases dissemination of colonising *S. pneumoniae* in *in vivo* models.

Following on from this work, we aim to determine how BC impacts the growth, behaviour and virulence of key bacteria associated with the exacerbation of COPD. Our current studies focus on the effects of air pollution exposure on biofilm formation and interaction with respiratory epithelial cells by non-typeable *H. influenzae* strain 375 and *M. catarrhalis* strain M61. Data shows that BC exposure is increasing epithelial cell adhesion, while also impacting biofilm formation, leading to a more fragile structure. This data indicates that COPD exacerbations may be altered in patients from highly polluted areas, due to the observed increase in colonisation phenotypes.

**P441**

# "Virulence mechanisms of pathogenic bacteria as targets for biodiscovery: Identification and characterization of anti-virulence molecules from marine sponges"

Neyaz A Khan[1], Laurence Jennings[2], Daniel Rodrigues[2], Laura Gallagher[3], Olivier P Thomas[2], James P O'Gara[3], Grace McCormack[4], Aoife Boyd[1]
[1]Pathogenic Mechanism Group, Discipline of Microbiology, National University of Ireland, Galway, Galway, Ireland. [2]Marine Biodiscovery, School of Chemistry and Ryan Institute, National University of Ireland, Galway, Galway, Ireland. [3]Department of Microbiology, School of Natural Sciences, National University of Ireland, Galway, Galway, Ireland. [4]Zoology, Ryan Institute, School of Natural Sciences, National University of Ireland, Galway, Galway, Ireland

## Abstract

Multi-drug resistant (MDR) pathogenic bacteria have become a global threat as traditional antibiotics become ineffective against infectious microbes. A promising approach for the development of next generation anti-microbials is to shift their target from bacterial death to inhibiting virulence. Hence, this channels our attention to anti-virulence molecules (AVMs) which impede virulence traits. The marine environment is an excellent source for bioactive molecules and AVMs. In this research, we screened Irish coastal sponges in search of novel AVMs against clinically relevant pathogens. Marine sponges are a potential reservoir of AVMS, as they have co-evolved along with their bacterial symbionts, at the same time defending themselves against bacterial pathogens. Out of 28 sponge species, fractions from 5 species inhibited biofilm formation of Methicillin Susceptible *Staphylococcus aureus*. Four of these fractions also decreased biofilm formation of Methicillin resistant *S. aureus*. The sponge fractions did not negatively affect bacterial growth or viability indicating a biofilm-specific effect. At a concentration of 100 µg/ml the fractions reduced biofilm by 76-90%, with the most active fraction resulting in the total absence of biofilm.,. Moreover, fractions from six different sponge species reduced biofilm formation of *Listeria monocytogenes* Purification and identification of the active compounds and their structure elucidation through Nuclear Magnetic Resonance (NMR) are ongoing.

**P442**

# Development of LAMP assays for the detection of key AMR targets in animal faeces and water samples

Marwa M. Hassan[1], Arnoud H. M. van Vliet[1], Owen Higgins[2], Liam P. Burke[3,4], Louise O'Connor[3,4], Dearbháile Morris[3,4], Terry Smith[2], Roberto M. La Ragione[5]
[1]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, United Kingdom. [2]Molecular Diagnostics Research Group, School of Natural Sciences, National University of Ireland Galway, Galway, Ireland. [3]Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, National University of Ireland Galway, Galway, Ireland. [4]Centre for One Health, Ryan Institute, National University of Ireland Galway, Galway, Ireland. [5]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey,, United Kingdom

## Abstract

Antimicrobial resistance (AMR) is a global issue affecting both human and animal health. The over-use and miss-use of antibiotics is the main driver of AMR, and therefore better diagnostics and interventions are urgently required. Rapid detection of AMR targets is crucial for identifying appropriate treatments, monitoring outbreaks and preventing the further spread of AMR. Loop-mediated isothermal amplification (LAMP) is a rapid diagnostic platform for the detection of nucleic acid targets without the need for thermal-based machines. This study aimed to develop a panel of LAMP assays to rapidly detect AMR bed-side and/or pen-side. LAMP primers were designed to specifically target plasmid-mediated colistin resistance (mcr-1), KPC-mediated carbapenem resistance (KPC) and oxacillin-hydrolysing β-lactamases (OXA-48 and OXA-23 genes). Mcr-1, KPC, OXA-48 and OXA-23 LAMP assays were tested and optimised to detect the targeted conserved sequences. All LAMP assays successfully detected the target genes within less than 5 min with excellent sensitivity and specificity against tested all Gram-positive and -negative strains. The LAMP assays demonstrated a detection limit of approximately 1 pg and 10 pg DNA using fluorescence and colorimetric detection, respectively. The assays will now be validated in the field and implemented as bed-side and/or pen-side diagnostics to facilitate the rapid detection of AMR.

## P443

# Antibacterial Action of Visible 405-nm light for Bacterial Reduction in Blood Plasma.

Caitlin Fiona Stewart[1], Heather Ralston[2], Ruairidh MacPherson[1], Mark Wilson[1], Scott MacGregor[1], Chintamani Atreya[3], Michelle Maclean[1,2]
[1]The Robertson Trust Laboratory for Electronic Sterilisation Technologies, University of Strathclyde, Glasgow, United Kingdom. [2]Department of Biomedical Engineering, University of Strathclyde, Glasgow, United Kingdom. [3]Office of Blood Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, USA

## Abstract

The introduction of risk prevention measures, such as blood screening and donor deferrals have dramatically reduced the incidence of transfusion-transmitted viral infections. Nevertheless, bacterial contamination of blood transfusion products remains a concern to patient health, and a range of pathogen reduction technologies have been developed to reduce this risk. Visible violet-blue light, in the region of 405-nm, has recently demonstrated potential for *in situ* treatment of *ex vivo* stored plasma and platelet products, without the need for additional photosensitizers.

This study assessed the broad-spectrum efficacy of 405-nm light against a range of bacteria implicated in transfusion-transmitted infections: *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae* and *Yersinia enterocolitica*. Plasma was seeded with clinically-relevant low-level bacterial contamination ($10^2$-$10^3$ $CFUmL^{-1}$) and exposed to a 405-nm light dose of 360 $Jcm^{-2}$ (1-hr at 100$mWcm^{-2}$) using a small-scale exposure system. Broad spectrum antibacterial efficacy was observed, with 99.0 – 100% inactivation achieved for all bacterial species tested. Bacterial inactivation tests were then scaled-up to expose large volumes of prebagged plasma seeded with *S. aureus* at ~$10^3$ $CFUmL^{-1}$, to 22$mWcm^{-2}$ 405-nm light, under agitation, (≤396 $Jcm^{-2}$). Successful bacterial inactivation was observed using the large-scale exposure system, with a dose of 238 $Jcm^{-2}$ (3-hr at ~22$mWcm^{-2}$) achieving complete (3.5-$\log_{10}$) reductions in prebagged bacterial-seeded plasma (P=0.001). Results from this study support further development of visible 405-nm light technology as a bactericidal tool for application in transfusion medicine.

*This abstract reflects the views of the author and should not be construed to represent FDA's views or policies.*

## P444

# Sepsis outcome in pediatric patients.

Svetlana Kolesnichenko, Alyona Lavrinenko, Lyudmila Akhmaltdinova, Elizoveta Kalmbach, Irina Kadyrova
Karaganda Medical University, Karaganda, Kazakhstan

## Abstract

Background. Pediatric patients are especially susceptible to infectious diseases and sepsis. Sepsis is one of the leading causes of death among the child population. Purpose of the study: to analyse and compare the characteristics of children of different ages with a diagnosis of sepsis and to determine predictors associated with an unfavorable outcome.
Methods. A retrospective descriptive study of the etiological structure and mortality among children diagnosed with sepsis for 2018-2020 was carried out in Karaganda (Kazakhstan). The children were divided into 5 age groups. The following parameters were analyzed: age, sex, primary loci of infection, infectious agent and outcomes. Statistical analysis was carried out in SPSS Statistics 20 software, using logistic regression.
Results. As a result of the study, it was determined that infants (1 to 11 months) with a diagnosis of sepsis were 2.277 (OR) times more likely to have a fatal outcome, p = 0.032. Analysis of unfavorable outcomes and the primary focus of infection relationship showed: children with intra-abdominal primary loci 2.17 (OR) times more often had negative outcome, p = 0.032. Children with *Enterococcus spp.* isolated from the blood were 4.34 (OR) times more likely to have a fatal outcome, p = 0.012.

Conclusions: According to the study, infants were identified as most vulnerable group of children, who more often had an unfavorable outcome. Intra-abdominal infection, which caused sepsis, more often than others led to a negative outcome in children of different age groups. In most cases, children with blood-derived *Enterococcus spp.* had fatal outcome.

## P445

# Comparative analysis of thymoquinone to amphotericin B in the pursuit of efficacious antifungal treatments utilising bacterial cellulose as a carrier.

Sam Swingler[1], Abhishek Gupta[1], Wayne Heaselgrave[1], Hazel Gibson[1], Marek Kowalczuk[2], Grazyna Adamus[2], Iza Radecka[1]
[1]University of Wolverhampton, Wolverhampton, United Kingdom. [2]Polish Academy of Sciences, Zabrze, Poland

## Abstract

Bacterial cellulose produced by *Komagateibacter xylinus* has been shown to possess a multitude of properties which make it innately useful as a next-generation biopolymer to be utilised as a wound dressing. The current study demonstrates the creation of a pharmaceutically active wound dressing by loading novel antifungal agents into the biopolymer hydrogel. Amphotericin B (AmB) is known to be highly hepatotoxic which reduces its appeal as an antifungal drug, especially in patients who are immunocompromised. This, coupled with an increase in antifungal resistance has seen a surge in fungal wound infections in patients who are immunodeficient as a result of chemotherapy, disease or injury. The purpose of this study was to compare the efficacy of the monoterpene, thymoquinone (TQ) against amphotericin B (AmB). The cytotoxicity of both compounds was tested against HeP-2 cell lines to establish the dose at which the compounds became inhibitory to cellular growth. Antifungal activity was conducted via Clinical & Laboratory Standards Institute (CLSI) M38 and M27 assays. The cytotoxicity assays where then ran in parallel to minimum fungicidal concentration ($MFC_{90}$) assays to determine at which concentration the compounds were active against disease causing fungal species (*Candida auris, Candida albicans, Aspergillus fumigatus* and *Aspergillus niger*). Amphotericin B had a breakpoint $MFC_{90}$ of 1-2 µg/mL with cytotoxic effects being observed at 98 µg/mL while thymoquinone had a breakpoint $MFC_{90}$ of 1.5 mg/mL with cytotoxic effects being observed at 30 mg/mL.

**P446**

# An attenuating role for blood-plasma conditioning films and selected blood plasma proteins on the adhesion forces of Staphylococcus aureus to central venous catheters as revealed by single-cell force spectroscopy.

Gubesh Gunaratnam[1], Christian Spengler[2], Simone Trautmann[3], Philipp Jung[1], Johannes Mischo[2], Ben Wieland[1], Carlos Metz[4], Sören L. Becker[1], Matthias Hannig[3], Karin Jacobs[2], Markus Bischoff[1]
[1]Institute for Medical Microbiology and Hygiene, Saarland University, Homburg, Germany. [2]Experimental Physics, Saarland University, Saarbrücken, Germany. [3]Clinic of Operative Dentistry and Periodontology, Saarland University, Homburg, Germany. [4]Department of Internal Medicine V, Pneumology and Intensive Care Medicine, Saarland University, Homburg, Germany

## Abstract

Background: Bacterial biofilms formed by *S. aureus* on implanted medical devices pose a serious risk to the patient. Especially when formed on medical devices that are inserted into the blood system, such as the central venous catheter (CVC), life-threatening infections can emerge.

Methods: We used single-cell force spectroscopy to quantify the adhesion forces of single, viable *S. aureus* cells on CVC tubings in presence and absence of human blood plasma (HBP). Additionally, we intended to clarify the role of the HBP main proteinaceous factors serum albumin (HSA) and fibrinogen (Fg) on the adhesion process.

Results: Our studies revealed that *S. aureus* adheres to naïve CVC tubings with low nN forces (2-7 nN). However, the forces dropped dramatically when the CVC tubing was preincubated with HBP, or when CVCs were probed after explantation from hospitalized patients. Subsequent investigations with HSA, the by far most abundant protein found in HBP, revealed that the strength of *S. aureus* to attach to the CVC tubing dropped to a similar extent, as has been seen with HBP. Interestingly, a clear reduction in the adhesion forces between *S. aureus* and the CVC tubing was also observed, when the CVC tubing was precoated by Fg, contrary to the assumed promoting function of Fg for *S. aureus* adhesion to implanted medical devices.

Conclusions: Our findings indicate that a precoating of catheter tubing with HSA might help to reduce the colonization risk of the CVC with *S. aureus* during the insertion process.

**P447**

# Identification of bacterial and human proteins directly from infected wounds of *in vitro* 3D skin models and intact *ex vivo* human skin: Towards *in situ* diagnosis

Jana Havlikova, Robin C. May, Iain B. Styles, Helen J. Cooper
University of Birmingham, Birmingham, United Kingdom

## Abstract

The ESKAPE pathogens represent a group of opportunistic and antibiotic resistant microbes responsible for nosocomial infections including those of skin. Currently, microbial identification takes hours or days. We have recently demonstrated that liquid extraction surface analysis mass spectrometry (LESA MS) allows for identification of bacterial species directly from the sample within minutes. Here we report the application of this to *in vitro* and *ex vivo* skin infection models.

*S. aureus* NCTC13435, *S. aureus* MSSA476, *K. pneumoniae* KP257 and *P. aeruginosa* PS1054 were used to infect wounded *in vitro* 3D skin models and incubated for 24, 48, 72 and 96 hours at 37°C, 5% $CO_2$ and >95% RH. *Ex vivo* human skin samples from HBRC were incubated at 37°C, 5% $CO_2$ and >95% RH. Samples were analysed by LESA MS on a Thermo Orbitrap Elite mass spectrometer.

Analysis of the samples resulted in identification of 7 human and 10 bacterial proteins after 96 hours of incubation. After 24 hours, only human skin proteins were identified, while after 48 hours, at least one bacterial protein was identified for every species tested. The protein d-hemolysin was observed for both *S. aureus* strains. The sequence differs between the two strains, resulting in a mass difference detectable by MS which could potentially be used as a biomarker. Experiments performed on the *ex vivo* human skin resulted in the mass spectra similar to those obtained from the Labskin samples. Optimized LESA MS parameters led to the identification of two skin proteins including ubiquitin and psoriasin.

## P448

# Comparison of the Antimicrobial Efficacy and Germicidal Efficiency of 405-nm Light for Surface Decontamination

Lucy Sinclair, Scott MacGregor, Michelle Maclean
University of Strathclyde, Glasgow, United Kingdom

## Abstract

**BACKGROUND:** The persistence of infectious organisms on hospital surfaces presents a significant challenge to healthcare environments. Low irradiance visible 405-nm light has recently been developed as a method for environmental decontamination, with studies demonstrating successful reductions of environmental bacteria in wards and operating theatres. This study investigates the antimicrobial efficacy of 405-nm light for decontamination of surfaces, and how the dose-response kinetics are affected by use of differing light irradiances.

**METHODS:** Surface-seeded *Staphylococcus aureus* and *Pseudomonas aeruginosa* (selected as model Gram-positive and Gram-negative species) were exposed to increasing doses of 405-nm light (≤ 90 $Jcm^{-2}$) at three discrete irradiances (0.5, 5 and 50 $mWcm^{-2}$). For both species, inactivation kinetics at each respective irradiance was established and susceptibility at equivalent light doses compared.

**RESULTS:** Results demonstrate increased bacterial susceptibility to 405-nm light inactivation when exposed at lower irradiance treatments. For both species, 3 $Jcm^{-2}$ was required when exposed using 0.5 $mWcm^{-2}$ irradiance to achieve significant bacterial inactivation ($P < 0.05$; 26.7-73.7% reduction). When exposed at 5 $mWcm^{-2}$, double the energy (6 $Jcm^{-2}$) was required to achieve similar reductions. Exposure at the highest irradiance (50 $mWcm^{-2}$) required 3-5 times greater dose (9-15 $Jcm^{-2}$) to achieve similar reductions to the lowest irradiance tested (0.5 $mWcm^{-2}$).

**CONCLUSION:** This study provides evidence of the enhanced germicidal efficiency of low irradiance 405-nm light, highlighting its efficacy for continuous environmental decontamination applications. Further investigation into the photo-chemical inactivation mechanisms will be crucial for its optimisation for a range of infection control applications.

## P450

# Salmonella SpvB induces caspase-independent cell death in a mART-dependent manner

Mitchell Pallett, Romina Tocci, Camilla Godlee, Andrea Majstorovic, Elena Everatt, David Holden
MRC CMBI, Imperial College London, London, United Kingdom

## Abstract

*Salmonella enterica* serovar Typhimurium (STm), causes gastroenteritis and non-typhoidal extra-intestinal disease in humans. Virulence of STm requires the spv locus which is associated with bacteraemia and disease severity. The spv operon contains four genes *spvA-D* and a regulator. *spvB* encodes a mono ADP-ribosyl transferase (mART) which induces the depolymerisation of actin, cell rounding and cell detachment following its delivery into the host cytoplasm by the SPI-1 or SPI-2 T3SS. It is hypothesised that SpvB contributes to virulence through inducing caspase-dependent apoptosis via actin ADP-ribosylation. However, whether SpvB contributes to SPI-2 induced cytotoxicity and the mechanisms by which actin depolymerisation induce cell death remain unknown. Here we sort to characterise the cell death induced by SpvB and its potential role in SPI-2 induced cytotoxicity. We show that ectopic expression of SpvB or treatment with the actin polymerisation inhibitor Latrunculin A induces cell cycle arrest, cell rounding/detachment, JNK phosphorylation and caspase-independent cell death in a mART-dependent manner. We further show that chemical inhibition or deletion of the stress kinase MAP4K4 rescues cell death induced by actin depolymerisation. To map the cell death signalling pathway cell lines stably expressing shRNA targeting key proteins involved in caspase-independent cell death (JNK1-3, AIFM1 and ENDOG) were generated. Whilst cell death was observed to be EndoG-independent studies are ongoing to further map the cell death signalling pathway and the role of SpvB in SPI-2 induced cytotoxicity. Importantly, our data suggests that whilst actin depolymerisation can activate caspase-3, cell death is in fact caspase-independent and signals through MAP4K4.

**P451**

# *Clostridioides difficile* surveillance at the University Hospital in Košice reveals high prevalence of the ribotype 176

Katarína Čurová[1], Annamária Toporová[1], Marcela Krůtová[2], Martin Novotný[1], Ľuboš Ambro[1], Anna Kamlárová[1], Ján Hockicko[1], Leonard Siegfried[1]
[1]Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovakia. [2]Faculty of Medicine, Charles University in Prague and Motol University Hospital, Praha, Czech Republic

## Abstract

Background: *Clostridioides difficile* infection (CDI) has become a serious health problem worldwide in recent years, the severity of which lies in the ability to spread epidemically in hospitals and in frequent diseases relapses. CDI Surveillance was performed at the Louis Pasteur University Hospital in Košice from January to February 2020 to analyse the molecular characteristics of *C. difficile* (CD) isolates from local patients with CDI.

Methods: CDI was initially diagnosed using the *C. difficile* rapid test (for enzyme GDH and toxin A/B). A total of 36 stool samples (29 GDH and toxin positive, 7 GDH positive and toxin negative) were cultured anaerobically on selective media (Brazier´s agar). Culture was positive for CD in 31 samples. Bacterial DNA was extracted from all CD isolates. Genes *tcdA*, *tcdB*, *cdtA* and *cdtB* encoding toxin A, toxin B and binary toxin were detected by multiplex PCR and ribotypes of CD were analysed by capillary electrophoresis-based PCR.

Results: Molecular typing showed that toxin A as well as toxin B was confirmed in 30 of 31 isolates, binary toxin in 18 isolates. Ribotype 176, characterized by production of all 3 toxins, was the most prevalent and was detected in 18 isolates (58%). Toxin A and toxin B producing ribotypes 001, 014 and 020 were also confirmed.

Conclusion: The high incidence of epidemic ribotype 176 with higher capacity to spread in a hospital setting emphasises the need to implement strict epidemic measures and the importance of implementing continuous surveillance programmes for CDI in Slovakia.

## P452

# The RNA-binding protein PNPase is a novel regulator of biofilm formation and virulence in *Listeria monocytogenes*

Ana Patrícia Quendera, Cecília Maria Arraiano, José Marques Andrade
ITQB NOVA – Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal

## Abstract

Bacterial biofilms provide a stress-enduring environment that makes bacteria more resilient to both the immune system and antibiotics. Biofilms constitute, thus, a bacterial survival strategy, which plays an important role in pathogenesis. Biofilm-related diseases are typically persistent infections, and a challenge for medical treatment. The knowledge of novel biofilm regulators may contribute to develop new strategies to fight microbial infections.

Here we show that the RNA-binding protein PNPase, a widely conserved 3'-5' exoribonuclease, is a novel biofilm regulator in the human pathogen *Listeria monocytogenes*. Inactivation of PNPase results not only in reduced formation of biofilm as it strongly affects its morphology. This suggested that PNPase could regulate biofilm matrix, a multicomponent substance that provides the structural support of the biofilm. Indeed, PNPase was found to affect the expression of polysaccharides, a major component of extracellular polymeric substances (EPS), with the Δ*pnp* mutant showing reduced sugar levels. This correlates with a less stable and less resistant biofilm produced by PNPase-deficient bacteria, which is more susceptible to antibiotic treatment. An altered EPS composition may further affect bacterial motility, and our results clearly showed that PNPase mutants are severely impaired in swimming motility. Furthermore, infection assays in different mammalian cell lines confirmed that inactivation of PNPase leads to the severe attenuation of *Listeria monocytogenes* pathogenicity.

Overall, our results show that PNPase is a novel regulator of biofilm formation and eukaryotic cellular invasion. This work provides new evidence that PNPase is an attractive target to the control of bacterial infection.

## P453

# Dissecting the molecular pathogenesis of *Legionella* spp. in human lung models

Flávia Viana[1], Oisín Cappa[1], John Stegmayr[2], David Simpson[1], Darcy Wagner[2], Cecilia O'Kane[1], Gunnar N. Schroeder[1]
[1]Wellcome-Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, United Kingdom. [2]Lung Bioengineering and Regeneration, Department of Experimental Medical Sciences, Wallenberg Centre for Molecular Medicine, Stem Cell Center, Faculty of Medicine, Lund University, Lund, Sweden

## Abstract

Acute respiratory infections are a leading cause of disease worldwide. Opportunistic infections by pathogens such as *Legionella* spp. increasingly contribute to this burden, a trend closely linked to growing susceptible populations including the elderly, immunosuppressed patients and those with underlying respiratory conditions. *Legionella* are environmental bacteria, which upon accidental inhalation can cause a severe pneumonia called Legionnaires' disease (LD); bacteria replicate in alveolar macrophages and host immune status determines disease progression. However, the underlying mechanisms governing susceptibility remain elusive as current infection models fail to recapitulate hallmarks of human disease and to elucidate the clinical predominance of a single isolate.

To mimic human lung *Legionella* infection we have established a new model using human precision-cut lung slices (hPCLS). These retain nearly intact structural, cellular and physiological integrity of the native lung, making them an outstanding platform to address human respiratory infections.

Our results indicate that in hPCLS *Legionella* infects and proliferates in macrophages and other cells. 10x single cell transcriptomics data show that hPCLS retain a remarkable cellular complexity highlighted by the identification of over 40 cell types (e.g. macrophages, ATI and II epithelial cells, Capillary Aerocytes, Basophils/Mast, NK and T cells) and that response to infection occurs at whole-tissue level. Additionally, infection of hPCLS with different *Legionella* isolates points to different capacities to infect and thrive in human lung tissue.

These results highlight hPCLS as a model for studying *Legionella* infection in the human lung that can provide unique insight into the molecular and cellular events underlying LD.

**P454**

# Probing mycobacterial metabolism in tuberculosis and leprosy to identify vulnerable metabolic nodes for drug development

Khushboo Borah[1], Martin Beyß[2], Karina Girardi[3], Tom Mendum[1], Flavio Lara[3], Katharina Nöh[2], Dany Beste[1], Johnjoe McFadden[1]
[1]University of Surrey, Guildford, United Kingdom. [2]Forschungszentrum Jülich,Institute of Bio- and Geosciences, IBG-1: Biotechnology, Jülich, Germany. [3]Instituto Oswaldo Cruz, Rio de Janeiro, Brazil

## Abstract

Metabolism of pathogens in infectious diseases is important for their survival, virulence and pathogenesis. Mycobacterial pathogens successfully scavenge multiple host nutrient sources in the intracellular niche. It is therefore important to identify the intracellular nutrient sources and their metabolic fates in these pathogens. Metabolic phenotype of an organism is defined by metabolic fluxes. We quantified *in vivo* fluxes of the pathogens and probed host-bacterial metabolic cross talks in tuberculosis (TB) and leprosy using systems-based strategies and techniques of isotopic labelling, metabolic modelling and metabolic flux analysis (MFA). We show that the TB pathogen metabolizes a number of carbon and nitrogen sources in human macrophages and identified vulnerable nodes such as glutamine and serine biosynthesis as potential drug targets. *Mycobacterium leprae*, the leprosy causing pathogen, uses host cell glucose in infected schwann cells and the enzyme, phoenolpyruvate carboxylase is a potential drug target. Our research provides an understanding of the intracellular diets and metabolism of these important human pathogens and identified vulnerable metabolic nodes that can be used for developing innovative chemotherapies in TB and leprosy.

## P455

# Recent insights into the structure-function relationship of the inverse autotransporter intimin

Julia Weikum[1,2], Sai Priya Sarma Kandanur[2], J. Preben Morth[1,2], Jack C. Leo[3,2]
[1]Technical University of Denmark, Lyngby, Denmark. [2]University of Oslo, Oslo, Norway. [3]Nottingham Trent University, Nottingham, United Kingdom

### Abstract

Intimin is an essential adhesin of attaching and effacing pathogens and the prototype of the type Ve secretion systems, also termed inverse autotransporters. We have solved the crystal structure of the two C-terminal immunoglobulin (Ig)-like domains in the passenger (extracellular region), D00 and D0. While D0 is structurally very similar to the already solved D1 and D2 domains of intimin, D00 is more divergent. The connector between D00-D0 forms an S-shaped hydrophobic loop that appears to confer a rigid orientation to the D00-D0 tandem. In contrast, the connections between D0-D1 and D1-D2 are flexible. Simulations show that the rigid connector between D00-D0 increases the reach of the adhesin, whereas the flexibility between D0, D1 and D2 domains allows the distal adhesive D3 domain to adopt the optimal orientation for receptor binding.

The intimin passenger is exported through the outer membrane via a hairpin-like intermediate. Though the hairpin model is accepted as the mechanism for autotransport, the formation of the hairpin is not understood. We addressed this by introducing mutations into the β-barrel domain that would disrupt conserved interactions between the linker (connecting the passenger and β-barrel) and residues on the luminal side of the β-barrel. Most of these mutations did not have a significant effect. However, disrupting a small antiparallel β-sheet at the extracellular side of the β-barrel, formed between two loops and the linker, had a drastic effect on the stability surface exposure of the protein, demonstrating a key role for this β-sheet in the inverse autotransport process.

## P456

# Development of Pseudomonas aeruginosa in growth media replicating the conditions of the upper and lower respiratory tract

Dilem Ruhluel[1], Dr Siobhan O'Brien[2], Dr Jo Fothergill[1], Dr Daniel Neil[1]
[1]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [2]Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom

## Abstract

The ability of *Pseudomonas aeruginosa* (Pa) to establish chronic infection in the cystic fibrosis (CF) lung result from a process of bacterial adaptation to the respiratory environment. Drivers of adaptation to the lung are poorly described. Robust *in vitro* models for performing evolution studies with Pa are also not currently available.

The respiratory tract is a heterogenous environment, with the nasopharynx and sinuses having distinct properties from the lungs. These differences may shape Pa adaptation and evolution. However, currently available *in vitro* models focus on the lung environment, with less consideration given to upper airway niches that are often early colonisation sites for respiratory bacterial pathogens, including Pa.

In respiratory microbiology, animal models have been widely used to bridge the gap between *in vitro* systems and the clinical scenarios. In addition to being costly and hard to maintain, they do not allow the upper airways and lower airways to be studied in isolation. It is desirable to be able to do this, as it is proposed that Paadaptation to the upper airway facilitates the establishment of lung infection in CF.

We have developed a suite of culture media reproducing key features of the upper and lower airways in health and altered environment in CF. The media will be used to perform experimental evolution with Pa, to study alterations in bacterial genotype and phenotype during growth in each media. Here, I present preliminary data aiming to define the composition of these media, showing differences in bacterial growth and biofilm formation.

**P457**

# Massively parallel transposon mutagenesis identifies temporally essential genes for biofilm formation in *Escherichia coli*

Emma Holden[1], Muhammad Yasir[1], Keith Turner[1], John Wain[1,2], Ian Charles[1,2], Mark Webber[1,2]
[1]Quadram Institute, Norwich, United Kingdom. [2]Norwich Medical School, Norwich, United Kingdom

## Abstract

Biofilms complete a lifecycle where cells aggregate, grow and produce a structured community before dispersing to seed biofilms in new environments. Progression through this lifecycle requires controlled temporal gene expression to maximise fitness at each stage. Previous studies have focused on the essential genome for the formation of a mature biofilm, but here we present an insight into the genes involved at different stages of biofilm formation. We used TraDIS-*Xpress*; a massively parallel transposon mutagenesis approach using transposon-located promoters to assay expression of all genes in the genome. We determined how gene essentiality and expression affects the fitness of *E. coli* growing as a biofilm on glass beads after 12, 24 and 48 hours. A selection of genes identified as important were then validated independently by assaying biofilm biomass, aggregation, curli biosynthesis and adhesion ability of defined mutants. We identified 48 genes that affected biofilm fitness including genes with known roles and those not previously implicated in biofilm formation. Regulation of type 1 fimbriae and motility were important at all time points. Adhesion and motility were important for the early biofilm, whereas matrix production and purine biosynthesis were only important as the biofilm matured. We found strong temporal contributions to biofilm fitness for some genes including some which were both beneficial and detrimental depending on the stage at which they are expressed. This work provides new insights into the requirements for successful biofilm formation through the biofilm life cycle and demonstrates the importance of understanding expression and fitness through time.

## P458

# Development of a CRISPR interference system in *Campylobacter jejuni*

Ruby Coates, Andrew Grant, Andres Floto, Julian Parkhill
University of Cambridge, Cambridge, United Kingdom

## Abstract

*Campylobacter spp.* are the leading cause of bacterial food-borne illness in humans worldwide, with *Campylobacter jejuni* responsible for 80% of these infections. There is no current vaccine and antibiotic resistance is emerging. There is an urgent need to understand fundamental *C. jejuni* biology for the development of new strategies to prevent and treat infections. The range of molecular tools available to regulate gene expression in *C. jejuni* is limited, which impacts studies into the function of essential and conditionally essential genes. My project aims to address this by applying a CRISPR-based interference system known as CRISPRi in *C. jejuni* as a means to control gene expression and thereby investigate gene function. To validate the CRISPRi system in *C. jejuni*, I have paired the dCas9 and sgRNA backbone from the *Streptococcus pyogenes* CRISPRi system with several *C. jejuni*-derived promoters to develop a series of CRISPRi constructs targeting several genes. Through rigorous sgRNA target design I have successfully targeted and repressed expression of the endogenous arylsulphatase (AstA) enzyme, as well as achieving partial repression of expression of the regulatory flagellar protein FlgR in two clinically relevant *C. jejuni* strains. This is the first report of a CRISPRi system for *Campylobacter*.

**P459**

# Characterisation of the Rh50 protein from the ammonia-oxidising bacterium Nitrosomonas europaea.

Adriana Bizior[1], Gordon Williamson[1], Gaëtan Dias Mirandela[2], Mélanie Boeckstaens[2], Paul Hoskisson[1], Anna Maria Marini[2], Ulrich Zachariae[3,4], Arnaud Javelle[1]
[1]Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom. [2]Biology of Membrane Transport Laboratory, Department of Molecular Biology, Brussels, Belgium. [3]Physics, School of Science and Engineering, Dundee, United Kingdom. [4]School of Life Sciences, Dundee, United Kingdom

## Abstract

The transport of ammonium through the cell membranes, an essential process in all kingdoms of life, is accomplished by the ubiquitous Amt/Mep/Rh superfamily of proteins. The functional context of Amt/Mep and Rh transporters is diverse: bacteria, fungi, and plants use Amt/Mep proteins to scavenge ammonium for biosynthetic assimilation, whereas mammals use the Rh proteins for ammonium detoxification in erythrocytes, kidney, and liver tissues. While RH50 genes are widespread in eukaryotes they are present in some prokaryotes: an example is a chemolithoautotroph *Nitrosomonas europaea* which gains all its energy from the oxidation of ammonia to nitrate.

While Amt/Mep/Rh proteins have divergent physiological functions, they are structurally very similar, which raises the important question about the universality of the transport mechanism. We have recently proposed an elegant new model for the mechanism of electrogenic ammonium transport in bacteria Amt protein: after deprotonation of $NH_4^+$ at the periplasmic side of the transporter, a previously undiscovered polar conduction route enables $H^+$ transfer into the cytoplasm. A parallel pathway, lined by hydrophobic groups within the protein core, facilitates the simultaneous transfer of uncharged $NH_3$. In this context, we propose to elucidate at the molecular level the mechanism of ammonium translocation through rhesus protein from *Nitrosomonas europaea* and establish whether there is a universal mechanism for biological ammonium transport. Beyond the elucidation of a central biological process, this work has important medical implications, as some Rh mutations have been associated with human pathologies. We propose to demonstrate how specific Rh mutations affect the activity of the protein to establish the relationship between Rh malfunction and the associated diseases.

## P460

# Chance and Necessity: Evolution guided antibiotic improvement and discovery

John Tyson Munnoch[1], Leena Kerr[2], Paul Hoskisson[1]
[1]University of Strathclyde, Glasgow, United Kingdom. [2]Institute for Life and Earth Sciences, Heriot Watt, Edinburgh, United Kingdom

## Abstract

*Streptomyces,* gram-postive, hyphal bacteria, are prolific producers of antibiotics that we exploit in human medicine (responsible for more than two-thirds used in the clinic). Understanding how these bacteria evolved and the regulation of antibiotic biosynthesis is important for key interest. Evolutionary adaption is driven by the accumulation of mutations, but the temporal dynamics of this process are difficult to observe directly in end-point analysis. Using a combination of Long-Term Experimental Evolution (LTEE), molecular genetics and genomics we have begun studying how the adaptive mutations shape the genome of *Streptomyces coelicolor* M1152, when and in what order. Our LTEE consists of growing M1152 in liquid culture, with a total of 6 lineages. Every 3 days, samples are passaged (1:100 dilution) into fresh media with a stock stored at -80°C. At generational milestones genomic phenotypic characterisation was carried out and DNA from the community was genome sequenced. Following the reintroduction of the actinorhodin biosynthetic gene cluster, representatives were sequenced to assess the impact on antibiotic production. We see both phenotypic and genotypic alterations. Increased hyphal fragmentation in liquid culture, markers of both developmental cessation and progression on solid-surface media, an accumulation of small mutations across the genome and examples of increased/reduced/loss of antibiotic production. Morphological adaption to liquid culture (increased fragmentation) is a desirable trait for fermentation. Developmental alterations are less linear than we would expect. These results are promising for the future of the experiment as we begin looking at the effects on antibiotic production and metabolic adaption.

## P461

# Aurodox: Teaching an Old Drug New Tricks

Rebecca E McHugh[1], Andrew Roe[2], Paul Hoskisson[1]
[1]Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom. [2]Institute of Infection, Immunity and Inflammation, University of GlasgowImm, Glasgow, United Kingdom

## Abstract

Aurodox, a specialised metabolite from the soil bacterium *Streptomyces goldiniensis* was discovered in 1972 and originally was investigated for its anti-staphylococcal and streptococcal properties. However, aurodox has been recently identified from large-scale compound screens as an inhibitor of the Enteropathogenic Escherichia coli (EPEC) Type III Secretion System (T3SS). Therefore, to gain an understanding of its mechanism of action and to assess the suitability of this molecule for repurposing as an anti-virulence compound multidisciplinary approach to understanding aurodox was used. Whole transcriptome analysis, cell infection and GFP-reporter assays were used to demonstrate that aurodox transcriptionally downregulates the expression of the Locus of Enterocyte Effacement (LEE) pathogenicity island- which encodes for the T3SS, acting via its master regulator, Ler. We have also observed these effects across other enteric pathogens carrying a homologous T3SS such as Enterohemorrhagic Escherichia coli (EHEC). Significantly, unlike traditional antibiotics, aurodox does not induce the production of shiga toxin. The biosynthesis of aurodox by *S. goldiniensis* was also investigated. Sequencing the whole genome of *S. goldiniensis* enabled the identification of the putative aurodox biosynthetic gene cluster (BGC). We have cloned and expressed this gene cluster in multiple heterologous hosts including *Streptomyces coelicolor* M1152 and can confirm this BGC is responsible for aurodox production. In-depth analysis of the BGC supports a model of a polyketide synthase pathway involving a combination of both cis and trans-Acyltransferases which synthesise the aurodox polyketide backbone. Furthermore, multiple aurodox resistance genes at distinct loci have been identified and their role in aurodox resistance has been explored.

## P462

# Disruption of the *mce* operon from *Streptomyces* affects spore resistance and results in precocious germination

Emily Addington[1], Laura Clark[2], Paul Hoskisson[1]
[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Oxford, Oxford, United Kingdom

## Abstract

*Streptomyces coelicolor* is a non-pathogenic soil saprophytic bacterium and is a model organism for antibiotic production. This species contains a single copy of a nine gene cluster known as the mammalian cell entry (*mce*) operon. This operon was originally characterised in *Mycobacterium tuberculosis* as an important virulence factor acting in invasion and survival within macrophages and encodes an ABC transporter for cholesterol import. As the function of the *mce* operon in *S. coelicolor* is currently unknown, this study aims to characterise the operon through deletion of the *mce* locus and resulting impact on bacterial morphology and survival. SEM images demonstrate that spores of a *mce* deletion mutant (*Δmce*) display a wrinkled, and 'fragile' phenotype, with spores appearing to germinate whilst on the spore chain. Germination assays show that spores of *Δmce* germinate earlier than WT *S. coelicolor*, and impression mounts indicate spore chains emerge earlier in the *Δmce* strain. As spores of *Δmce* possess an altered spore coat, it was hypothesised they might show reduced resistance to stressors. Heat kill assays show that deletion of the *mce* operon results in *S. coelicolor* spores which are less tolerant to temperatures of 60, 70, 80, 90 and 100°C compared to WT *S. coelicolor* spores. Heat activation of *Δmce* spores was also consistently absent at all temperatures tested. Spores of the *Δmce* strain are also less resistant to triclosan, and sulfobetaines. Preliminary results indicate that the *mce* operon of *S. coelicolor* may be a cholesterol importer, as *Δmce* shows reduced growth in the presence of cholesterol, compared to WT *S. coelicolor*.

## P463

# Understanding the role of pyruvate phosphate dikinase in *Streptomyces.*

Molly Keith-Baker[1], Steve Kendrew[2], Andrew Collis[2], Benjamin Huckle[2], Iain Hunter[1], Paul Hoskisson[1]
[1]University of Strathclyde, Glasgow, United Kingdom. [2]GlaxoSmithKline, Worthing, United Kingdom

## Abstract

To combat the global antimicrobial resistance crisis there is a need to develop new antibiotics and to increase the production of existing antimicrobials. Pyruvate kinase (Pyk) has been shown to be a good target for metabolic engineering to increase antibiotic production. Pyruvatephosphate dikinase (PPDK) usually catalyses the opposite reaction to Pyk and in contrast is reversible and occurs in a three-step process converting pyruvate, ATP and inorganic phosphate into AMP, PEP and diphosphate. There are two copies of Pyk in the majority of *Streptomyces* and interestingly, there are also two copies of PpdK, indicating that there are four potential biochemical routes to converting pyruvate to PEP. There are currently no studies involving the role of PPDK in actinobacteria, however our previous work has shown that one of the Ppdk enzymes in *S. coelicolor* is upregulated 30-fold prior to antibiotic production.

To investigate the role of PPDK in *Streptomyces,* knockdown mutants were developed using the CRISPRi /dCas9 technology as well over-expression of the proteins in WT strains. These strains exhibit both growth and developmental phenotypes. In doing this we hope to be able to better understand the role of PPKD in the primary metabolism of streptomycetes and the flow of metabolites around the pyruvate-phosphoenolpyruvate-oxaloacetate node of central metabolism under a range of conditions and to understand how this impacts the availability of precursor molecules for specialised metabolite production.

## P464

# Actin dynamics regulate proteasome homeostasis

Thomas Williams, Adrien Rousseau
University of Dundee, Dundee, United Kingdom

### Abstract

Cells require thousands of unique proteins to be in the right place, at the right time, in the right amounts and with the right modifications. They do this through several processes collectively known as protein homeostasis. TORC1 is a principal regulator of protein homeostasis, coordinating protein synthesis and degradation. The proteasome, composed of a core particle and one or two regulatory particles, degrades unwanted protein. Diverse stresses cause a protein homeostasis imbalance: inhibiting TORC1 and the misfolded/damaged protein load. Following TORC1 inhibition, proteasome regulatory particle assembly chaperone (RPAC) translation is increased and thus cells assemble more proteasomes to degrade the damaged and misfolded proteins, thereby restoring protein homeostasis.

Using yeast, we identify an endocytic protein, Ede1, that interacts with and is critical for translation of RPAC mRNA following TORC1 inhibition. We find two further endocytic proteins important for RPAC translation regulation. Mutants of these proteins cause altered Arp2/3 activity, and hence altered formation of actin patches/endocytic sites. We show that RPAC mRNA is transported on actin cables and interacts with actin patches. TORC1 inhibition depolarises the actin cytoskeleton, causing RPAC mRNA accumulation on actin patches concurrent with translation. We demonstrate Ede1 is essential for RPAC mRNA localisation regulation following rapamycin treatment.

This work shows that, upon actin depolarisation, RPAC mRNA is recruited to actin patches, likely by Ede1, and translation occurs. Actin regulation is therefore a key element of proteasome (and therefore protein) homeostasis.

## P465

# An intracellular copper delivery service in *Neisseria gonorrhoeae*

Samantha Firth[1], Karrera Djoko[1], Louisa Stewart[1], YoungJin Hong[1], Daniel Owen[1], Kevin Waldron[2], Alastair McEwan[3], Bostjan Kobe[3], Denis Thaqi[3]
[1]Durham University, Durham, United Kingdom. [2]Newcastle University, Newcastle upon Tyne, United Kingdom. [3]University of Queensland, St Lucia, Australia

## Abstract

The obligate human pathogen *Neisseria gonorrhoeae* colonises the microaerobic genitourinary tract. In these $O_2$-limited conditions, nitrite is used as a terminal electron acceptor for respiration, in a process known as denitrification. The central enzyme in this process is the nitrite reductase AniA, which is a copper-dependent enzyme. Interestingly, AniA is localised to the outer membrane, raising the question of how and where this enzyme acquires its copper cofactors. A random deletion mutagenesis screen identified a possible periplasmic factor for the delivery of copper into AniA, called AccA. *N. gonorrhoeae* knockout mutant strains lacking the *accA* gene fail to grow in microaerobic conditions and reduce $NO_2^-$, consistent with an inactive AniA. This phenotype is reversed when copper is supplemented to the growth media, suggesting that, in the absence of AccA, AniA is expressed as an *apo*-enzyme. Conserved Met and His residues in AccA are predicted to be involved in the binding and delivery of Cu to AniA. To probe the role of these residues, *N. gonorrhoeae* mutants expressing site-directed Met to Ala and His to Ala AccA variants, as well as a truncated variant lacking the Met- and His-rich C-terminal tail have been constructed. Bacterial growth under both Cu excess and limited conditions, and rates of $NO_2^-$ respiration have been subsequently measured. The results have allowed us to build a model of AniA active site assembly and general copper handling in the *N. gonorrhoeae* periplasm. In the future, blocking the copper transfer from AccA to AniA may provide a novel antibacterial strategy to kill multi-drug resistant *N. gonorrhoeae.*

## P466

# Localization dynamics of N-acetylglucosamine transporter, Ngt1 in *Candida albicans*

Kasturi Roy, Swagata Ghosh
University of Kalyani, Kalyani, India

## Abstract

The amino sugar N-acetylglucosamine (GlcNAc), widely present in multiple cell-surfaces is efficiently utilized by human pathogen *Candida albicans* at the sites of infection as a survival strategy in different host niches. GlcNAc import inside the cell is mediated by GlcNAc transporter, Ngt1. In this present study, to investigate the Ngt1 dynamics, we have checked the sensitivity of Ngt1 for GlcNAc, expression kinetics and dynamics of Ngt1, stability of Ngt1 in the presence of unrelated carbon source like, glucose and endocytic trafficking of Ngt1. For this study we have used epi-fluorescence microscopy, Western blot and Northern blot analysis. We have observed that Ngt1 expression is prolific and highly sensitive to even minute amount (<2 μM) of GlcNAc. Ngt1 maintains its turnover in the plasma membrane by sugar stimulated endocytosis via *UBI4* (polyubiquitin encoding locus) mediated ubiquitylation. Co-localization studies with different sub-cellular markers have revealed that Ngt1 follows a trafficking route via early endosome-late endosome-multi vesicular body (MVB)-Trans Golgi network for degradation. In conclusion, the study will provide a better understanding of endosomal trafficking for other membrane proteins in particular sugar transporters and also will open new therapeutic aspects in *Candida albicans.*

**P467**

# Investigating RNA polymerase regulation in the antibiotic producing *Streptomyces* bacteria.

Hayley Greenfield, Mark Paget, Stephen Hare
University of Sussex, Falmer, United Kingdom

## Abstract

Organisms belonging to the *Streptomyces* genus produce a vast number of useful secondary metabolites, including over half of all clinically relevant antibiotics. Recent genome sequencing, however, has revealed large clusters of genes in *Streptomyces* that are not expressed under standard growth conditions and that hold potential for the discovery of novel antibiotics. Several studies have shown that certain RNA polymerase mutations can increase antibiotic production, as well as stimulate the synthesis of previously undetected secondary metabolites. However, the basis of this is not understood and little is known about how RNA polymerase levels are controlled.

Our analysis of RNA -seq data from *S. coelicolor* and *S. venezuelae* revealed that the *rpoBC* operon, which encodes the large β and β' subunits of RNA polymerase, is subjected to a largely unstudied form of gene regulation known as reiterative transcription (RT) at the transcriptional start site. Mutagenesis of the promoter and untranslated region will be presented, which are starting to reveal how RT might control *rpoBC* expression in response to stress.

A greater understanding of how RNA polymerase levels are controlled in *Streptomyces*, might enable novel approaches towards the synthesis of novel inhibitory compounds and therefore contribute to overcoming the current antimicrobial resistance crisis.

## P469

# Back to soil: Awakening the production of cryptic antibiotics in *Streptomyces*

Jana Katharina Schniete, Joseph Paros, Lorena Fernández-Martínez
Edge Hill University, Ormskirk, United Kingdom

## Abstract

Most of the antibiotic biosynthetic gene clusters (BGCs) in *Streptomyces* are not expressed under laboratory conditions, however these clusters are maintained in the genomes, therefore indicating that they must play important roles in adaptation and survival within their ecological niches.

Understanding the global regulation patterns that affect transcription of antibiotic BGCs in soil conditions versus laboratory conditions allows us to identify novel routes for up- or down- regulation in order to trigger expression of these BGCs and therefore production of these antibiotics under controlled laboratory and fermentation conditions. Cryptic pathways represent an untapped resource in terms of new metabolites that could be very useful in the clinic if we can awaken their expression and production.

We are using RNA sequencing to analyse global transcriptional patterns of *S. venezuelae* and *S. rimosus* grown in sterile soil, non-sterile soil and standard laboratory solid medium, as well as using an unbiased metabolomics approach to identify compounds produced in soils. These results will enable the identification of global regulatory pathways that activate cryptic clusters in nature and lead to the production of these compounds in soil.

This will enable us to generate genetically modified strains engineered to increase the yield of both well characterised and cryptic clusters which encode potentially novel compounds under industrial fermentation conditions. It is worth noting that the transcriptional data may also help shed light on the complex regulation of the life cycle of these *Streptomyces* strains in an ecologically relevant environment.

## P470

# Activation of the Escherichia coli Rtc RNA repair system: a network of signalling pathways.

Leonor De Abreu Nunes, Pranav Sudheesh, Ioly Kotta-Loizou, Martin Buck
Imperial College London, London, United Kingdom

## Abstract

RtcR is a multidomain DNA-binding protein acting as the transcriptional activator of the *rtcBA* operon, which encodes the RNA cyclase RtcA and the RNA ligase RtcB, implicated in RNA repair. The Rtc system is activated following oxidative stress or antibiotics exposure. Here we focus on uncovering pathways leading to RtcR activation. Public databases were used to compile a dataset of Rtc-linked protein, DNA and RNA interactions in *Escherichia coli* K12. This information was then manually curated with literature-based annotations, specifying interaction type and detection methods. Cytoscape was used for network visualisation, and PantherDB for Gene Ontology (GO) enrichment analysis. A dataset of over 24K interactions, including enzymes, transcription factors, sigma proteins, messenger (m)RNAs and small non-coding (snc)RNAs, operons and transcription units, was generated and visualised. GO enrichment analysis revealed that cellular respiration, carbohydrate and cellular catabolism, organonitrogen, amide and alpha-amino acid biosynthesis, response to abiotic stimuli and stress are enriched biological processes in the network. Negative (but not positive) regulation of gene expression, transcription and macromolecule biosynthesis were also enriched. This network provides a broad overview of the interactions involving Rtc proteins and may allow through reverse genetics the identification of the molecules and pathways leading to the activation of the Rtc RNA repair system. In conclusion, this research provides insights into the cellular mechanism of RtcR activation, while establishing a global understanding of the macromolecular interactions and pathways linked to *E. coli* RNA repair by the Rtc system.

**P471**

# *Ruminococcus gnavus* GH98 substrate specificity to blood group A antigen contributes to mucin glycan foraging

Haiyang Wu[1], Emmanuelle Crost[1], David Owen[2,3], Wouter van Bakel[1], Ana Martinez-Gascuena[1], Martin Walsh[2,3], Jesus Angulo[4,5], Nathalie Juge[1]
[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Diamond Light Source Ltd, Didcot, United Kingdom. [3]Research Complex at Harwell, Rutherford Appleton Laboratory, Didcot, United Kingdom. [4]School of Pharmacy, University of East Anglia, Norwich, United Kingdom. [5]Departamento de Química Orgánica, Universidad de Sevilla, Sevilla, Spain

## Abstract

The human gut symbiont *Ruminococcus gnavus* displays a strain-specific repertoire of glycoside hydrolases (GHs) contributing to its spatial location in the gut. Sequence similarity networks showed that *R. gnavus* GH98 (*Rg*GH98) sequence fell in a cluster different from sequences of GH98 enzymes functionally characterised to date. We heterologously expressed and purified *Rg*GH98, and determined its substrate and linkage specificity. We showed that *Rg*GH98 is specific for blood group A antigen (BgA), as also confirmed by isothermal titration calorimetry (ITC) and saturation transfer difference (STD) NMR, revealing affinity for blood group A over blood group B and H antigens. The molecular basis of *Rg*GH98 specificity was further investigated using a combination of site-directed mutagenesis and X-ray crystallography. The crystal structure of the complex between *Rg*GH98 and BgA trisaccharide and *Rg*GH98 inactive mutant with BgA tetrasaccharide identified residues involved in *Rg*GH98 unique specificity. RNAseq and qPCR analysis showed that the gene encoding *Rg*GH98 is part of an operon that is overexpresssed *in vitro* when *R. gnavus* is grown on mucin as sole carbon source. We showed that *Rg*GH98 releases BgA trisaccharide from mucin and that pretreatment of mucin with *Rg*GH98 conferred other *R. gnavus* strains lacking this enzyme the ability to grow through BgA metabolism and access to the underlying mucin glycan chain. These data further support that the GH repertoire of *R. gnavus* strains enables them to colonise different nutritional niches in the gut and provide a source of enzymes with unique specificities for potential applications in diagnostic or therapeutics.

## P472

# The stressosome controls motility, growth and acid survival of *Vibrio vulnificus*

Laura Cutugno[1], Jan Pané-Farré[2], Conor O'Byrne[1], Aoife Boyd[1]
[1]Discipline of Microbiology, School of Natural Sciences, National University of Ireland, Galway, Ireland.
[2]Centre for synthetic Microbiology (SYNMIKRO) & Department of Chemistry, Philipps-University, Marburg, Germany

## Abstract

The stressosome complex mediates the stress response in bacteria. In Gram-positive bacteria the stressosome regulates $\sigma^B$ activity. In this work, attention is addressed to the stressosome in a Gram-negative that does not possess $\sigma^B$, *Vibrio vulnificus*. This bacterium populates estuarine waters and can cause infections in humans following consumption of seafood or wound exposure to seawater. The work genetically dissected the stressosome locus, made up of upstream and downstream modules, containing genes coding for the main stressosome proteins (*vvuR, S, T* and *X*) and a two-component regulatory system (*vvuD1* and *D2*) respectively, in order to characterise its role in *V. vulnificus.* Two mutants were successfully created, one lacking the whole upstream module (ΔRSTX) and the other lacking the sensor kinase of the downstream module (ΔD1). Phenotypic characterisation of the mutants ruled out a role for the stressosome in rich media but showed an effect on growth and survival in chemically defined media. Both mutants had a growth advantage in late log phase when compared to the wild type. Interestingly, the downstream module enhanced survival of the so-called suicide phenomenon, whereby bacteria kill themselves by acidifying the media to toxic levels through the metabolism of glucose into acetate. Moreover, the effect of these mutations on the motility of *V. vulnificus* were demonstrated. These results give the first insights on the *in vivo* role of the stressosome in modulating motility, growth and stress survival in Gram-negative bacteria.

**P473**

# How do *Streptomyces* coordinate DNA repair and cell division following DNA damage?

Kathryn Stratton[1], Matthew Bush[1], Govind Chandra[1], Kim Findlay[2], Susan Schlimpert[1]
[1]Department of Molecular Microbiology, John Innes Centre, Norwich, United Kingdom. [2]Department of Cell and Developmental Biology, John Innes Centre, Norwich, United Kingdom

## Abstract

DNA damage often results in a pause of cell division until damage is repaired. In bacteria, a widely conserved response to DNA damage is the SOS response which relies on two proteins: the multifunctional recombinase RecA and the transcriptional repressor LexA. Under DNA-damaging conditions, this response activates proteins involved in DNA repair and the inhibition of cell division which in most unicellular bacteria results in temporarily filamentous growth. However, it is unknown how naturally filamentous growing bacteria like *Streptomyces* cope with DNA damage and how DNA damage repair is coordinated with cell division.

To identify novel regulators of cell division in *Streptomyces* that specifically function during DNA damaging growth conditions, we investigated the global response of *Streptomyces venezuelae* to several genotoxic agents, including mitomycin C, ciprofloxacin and methane methylsulfonate. To this end we have performed ChIP-seq experiments to identify the LexA regulon in *Streptomyces* and conducted RNA-seq experiments to determine the global response to DNA damaging agents in the wildtype and a *recA* mutant. The combined analysis of the available data sets has allowed us to obtain a comprehensive overview about the genes involved in the SOS response in *Streptomyces* and further analysis will enable us to understand how damage repair and cell division are coordinated in these bacteria.

**P474**

# Exploring 2,7-anhydro-Neu5Ac for precise modulation of the human gut microbiota

Andrew Bell[1], Laura Vaux[1], Emmanuelle Crost[1], Emmanuele Severi[2], Gavin Thomas[3], Nathalie Juge[1]
[1]Quadram Institute, Norwich, United Kingdom. [2]University of Newcastle, Newcastle, United Kingdom. [3]University of York, York, United Kingdom

## Abstract

*Ruminococcus gnavus* is a prevalent member of the human gut microbiota and has a profound impact on health and disease. We recently unraveled *R. gnavus* pathway for the recognition and metabolism of sialic acid, a sugar found in terminal location of mucins. This unique pathway is based on *R. gnavus* ability to produce 2,7-anhydro-Neu5Ac from sialylated substrates using an intra-molecular *trans*-sialidase, the exquisite specificity of its sialic acid transporter for 2,7-anhydro-Neu5Ac and of an oxidoreductase enzyme capable of reversibly converting 2,7-anhydro-Neu5Ac to Neu5Ac once inside the cell. We showed that this pathway is critical to *R. gnavus* fitness and spatial adaptation in the gut. Here we further explored the effect of 2,7-anhydro-Neu5Ac on the growth of enteric pathogens and modulation of the gut microbiota *in vitro*.

We showed that enteric pathogens *Salmonella* Typhimurium and *Clostridium difficile,* that scavenge free Neu5Ac in the mucosal niche released by other gut symbionts, could not utilise 2,7-anhydro-Neu5Ac while they could grow to high level on Neu5Ac. In addition, 2.7-anhydro-Neu5Ac was shown to preferentially promote *R. gnavus* growth in *in vitro* colon models seeded with human faecal samples from donors and supplemented with 2.7-anhydro-Neu5Ac, Neu5Ac or 3'sialylactose, as monitored over time by qPCR. Combining this approach with 16S rRNA sequencing and taxonomic analysis revealed that a limited number of gut commensal bacteria were capable of metabolising 2,7-anhydro-Neu5Ac, in line with our *in silico* analyses, supporting its possible use as next generation prebiotics. Together these data could help develop alternative antimicrobial strategy to limit growth of pathogens in the gut.

**P475**

# A tryptophan 'gate' in the CRISPR-Cas3 nuclease controls ssDNA entry into the nuclease site, that when removed results in nuclease hyperactivity.

Liu He[1], Zoe Jelić Matošević[2], Damjan Mitić[3], Dora Markulin[3], Tom Killelea[4], Marija Matković[5], Branimir Bertoša[2], Ivana Ivančić-Baće[3], Edward Bolt[1]
[1]School of Life Science, University of Nottingham, Nottingham, United Kingdom. [2]Department of Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia. [3]Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia. [4]University of Nottingham, nottingham, United Kingdom. [5]Institute Ruđer Bošković, Zagreb, Croatia

## Abstract

Cas3 is a ssDNA-targeting nuclease-helicase essential for class 1 CRISPR immunity systems that has been utilized for genome editing along with a surveillance complex 'Cascade' in human cells. Cas3-DNA crystal structures show that ssDNA follows a pathway from superfamily 2 helicase domains through to a HD-nuclease active site, requiring protein conformational flexibility during DNA translocation. In genetic studies we had noted that the efficacy of Cas3 in CRISPR immunity was drastically reduced when temperature was increased from 30 $^{o}$C to 37 $^{o}$C, by unknown mechanism. Here using *E. coli* Cas3 proteins we show that inhibition of nuclease activity at higher temperature corresponds with measurable changes in protein structure. This effect of temperature on Cas3 was alleviated by changing a highly conserved tryptophan residue (Trp-406) into alanine. The Cas3$^{W406A}$ protein is a hyperactive nuclease that functions independently from temperature and from the interference effector Cascade. Trp-406 is situated at the interface of Cas3 HD and RecA1 domains that is important for maneuvering DNA into the nuclease sites. Molecular dynamics simulations based on the experimental data showed temperature-induced changes in Trp-406 positioning that either blocked or cleared the ssDNA pathway. We propose that Trp-406 forms a 'gate' for controlling Cas3 nuclease activity *via* access of ssDNA to the nuclease active site. The effect of temperature in these experiments may indicate allosteric control of Cas3 nuclease activity caused by changes in protein conformations. Our unearthing of hyperactive Cas3$^{W406A}$ may offer improved Cas3-based genetic editing in human cells, especially for large scale DNA deletion.

**P476**

# Characterisation of genes essential for growth of fish derived *S. agalactiae* in the presence of hydrogen peroxide

Morena Santi, Adam Blanchard, James Leigh, Sharon Egan
School of Veterinary Medicine and Science, University of Nottingham, Nottingham, United Kingdom

## Abstract

*Streptococcus agalactiae* or Group B Streptococcus (GBS), is a highly infectious zoonotic pathogen, responsible for streptococcosis in fish, meningitis and septicaemia in humans and mastitis in cattle. In fish, it manifests and septicaemia, resulting in high morbidity and mortality in a number of wild and farmed species, in particular tilapia, sea bass and rainbow trout. A population of GBS mutants were generated using the pGh9:*ISS1* insertional mutagenesis system, to identify conditionally essential genes required for survival in the presence of hydrogen peroxide, to investigate the role of reactive oxygen species in vitro. Comparative sequence analysis was performed on approximately 100,000 individual mutants grown in bacterial media in the presence and absence of hydrogen peroxide using the PIMMS transposon analysis platform. 44 genes were found to be required for growth in the presence of hydrogen peroxide, including genes involved in cell division, peptidoglycan formation and cell survival during oxidative stress. Among these were *PcsB*, an extracellular protein involved in peptidoglycan hydrolyses and cell division regulation, *SpxA*, which modulates cell survival during oxidative stress and has previously been linked to virulence in Streptococci species, *Ffh* involved in the secretion and insertion of membrane proteins and *Dltx* involved in d-Alanylation of teichoic acids involved in charge based regulation of the cell envelope and resisting the effects of positively charged host immune factors. This study highlights the potential use for transposon mutagenesis to rapidly identify potential vaccine targets for aquaculture allowing this growing industry to become more environmentally sustainable.

## P478

# Assessment of Different Substrates on a Microbial Community using an *Ex Vivo* Colonic Model

Cathy Lordan[1,2], Paul Ross[3], Paul Cotter[1,3]
[1]Teagasc, Fermoy, Ireland. [2]University College Cork, Cork, Ireland. [3]APC Microbiome Ireland, Cork, Ireland

## Abstract

The contribution of the gut microbiota to health and disease is becoming increasingly apparent due to developments in DNA sequencing and cultivation techniques. There has been a lot of focus on enhancing the growth of health beneficial microbes, especially bifidobacteria and lactobacilli, through the use of prebiotics, i.e., non-digestible food substrates which are selectively utilised by beneficial bacteria. Other bacteria, recently identified as a potentially new generation of health-promoting microbes, may also be targeted to enhance their growth. These include anaerobes such as *Akkermansia muciniphila, Faecalibacterium prausnitzii* and *Eubacterium rectale*. However, there remains much to be learned about the direct impact that different substrates have on the growth and activity of the diverse members of the gut microbiota. Following a series of laboratory-based studies with a variety of substrates, we employed an *ex vivo* colonic model to test a range of substrates in various combinations on a gut microbial community. These substrates include oligosaccharides as well as simple sugars. *Ex vivo* models provide a reproducible, rapid and inexpensive means of assessing the colonic microbiota. An anaerobic environment with controlled temperature and pH parameters was used in this study. Samples were obtained at 0h and 24h to establish the impact of these substrates before and after fermentation. Shotgun metagenomic sequencing was applied in order to unravel the composition and functional potential of this complex community. A combination of computational approaches including, strain-level taxonomic classification, metabolite profiling, and prediction of substrate utilisation, the ability to stimulate the growth of species of interest was assessed.

**P479**

# Strain-specific differences in cell surface glycosylation of the human gut symbiont *Ruminococcus gnavus*

Victor Laplanche[1], Luke Mitchell[1], Emmanuelle Crost[1], Cristina De Castro[2], Nathalie Juge[1]
[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]UNIVERSITA' DEGLI STUDI DI NAPOLI FEDERICO II, Napoli, Italy

## Abstract

*Ruminococcus gnavus* is an important member of the human gut microbiota associated with health and disease. There is a need to better understand the mechanisms underpinning its adaptation to the gut and impact on human health. We previously showed that the ability of *R. gnavus* to utilise mucin glycans is strain-dependent and associated with its glycosidase profile[1-3]. Here we hypothesised that differences in mucin glycan metabolism may influence *R. gnavus* cell surface glycosylation affecting its interaction with the host.

Three strains of *R. gnavus* isolated from healthy donors - two mucin glycan-foraging strains ATCC 29149 and 35913 and the non- mucin glycan-foraging strain E1 - were grown under strict anaerobic conditions on different carbon sources. We first used *in silico* analysis and flow cytometry lectin-binding assay, to screen carbohydrate epitopes on the cell surface of these strains. The lectin binding profiles were found to vary depending on the strain and on the carbohydrate source. *In silico* analysis revealed difference in the glucorhamnan biosynthesis gene cluster in the strains ATCC 29149, E1 and ATCC 35913 which may contribute the phenotypic analysis. We next carried out a detailed investigation of *R. gnavus* cell surface polysaccharide using GC-MS and $^{1}$H-NMR analyses. The analysis revealed strain-specific differences in the glucorhamnan structure in terms of number of rhamnose residues constituting the backbone and glucose residues in the sidechains.

Taken together these data may reflect variable immunomodulatory properties of *R. gnavus* strains associated to distinct nutritional niches in the gut.

Crost et al. [1]PLoS One. 2013; [2]Gut Microbes. 2016; [3]Wu et al. Cell Mol Life Sci. 2021

## P481

# The Rtc RNA repair system is linked to virulence in *Escherichia coli.*

Danai-Athina Irakleidi, Martin Buck, Ioly Kotta-Loizou
Imperial College London, London, United Kingdom

## Abstract

Antibiotic resistance is one of the biggest public health challenges of the 21st century. The Rtc RNA repair system is present in many pathogenic bacterial species, including the model organism and putative pathogen *Escherichia coli*, and may play a role in antibiotic resistance. Here we explore its physiological role during infection. Pathogenicity assays are being performed using the infection model *Galleria mellonella*, to study the phenotypes and survival rates following larvae infection with *E. coli* K12 and variants lacking *rtc* genes. Larvae infected with wild-type bacteria survive for up to 10 days as opposed to those lacking any of the *rtc* genes, which survive for up to 14 days on average. Complementation of the *rtc* gene deletions with wild-type Rtc proteins, but not functionally catalytic mutants, reverses the infection phenotype to that of wild-type. Similarly, viable larvae infected with wild-type or complemented bacteria score much lower in a health index scoring system than those infected with strains either lacking any of *rtc* genes or expressing catalytically inactive Rtc proteins. To further explore the importance of Rtc in infection, bacterial burden assays will be conducted to measure the bacterial load. The results so far suggest that bacteria with a fully functional Rtc system are more aggressive in the *G. mellonella* infection model. This outcome supports the notion that the Rtc RNA repair system is involved in bacterial virulence and opens the window for further research to understand the ways in which Rtc may contribute to resistance, with potential for broader health benefit.

## P482

# Bioinformatic Analysis for Iron-Uptake System of Campylobacter spp. in humans and chickens

Mariklairi Kiourkou, Julian Ketley, Natalie Barratt
University of Leicester, Leicester, United Kingdom

## Abstract

Asymptomatic *Campylobacter jejuni* colonisation is highly prevalent in chickens, however, it's a major cause of bacterial foodborne disease in humans. Iron is an essential co-factor in many physiological processes and *Campylobacter* strains employ several non-redundant iron acquisition systems for survival and colonization. The outer membrane receptors CfrA and CfrB are involved in iron acquisition from catecholamine siderophores and are required for colonisation of the chicken GI tract. In contrast, ChuAB are part of an uptake system, enabling the utilization of iron in haem and is not required for colonisation. This study aims to compare differences in the iron-uptake systems present in *Campylobacter* strains isolated from chickens or humans.

Analysis of *Campylobacter jejuni/coli* genome sequences in PubMLST (https://pubmlst.org/organisms/campylobacter-jejunicoli) added from the UK in 2018 reveals differences in sequence type (ST) and *chuA, chuB, cfrA and cfrB* between isolates from chicken (CI) or human (HI) sources. ST828 was the most common ST for the *C. coli* CI (45%) and ST827 (32%) for the HI. *C. jejuni* ST5136 was the most common of the HI (10%) and CI (13%), ST50 (8%) for the HI and ST21 (7%) for the CI. >95% of isolates contained *chuA* and *chuB*, we also found the Chu system to be highly conserved. For *cfrA* 81% of HI and 74% of CI contained the gene, and 92% of HI and 96% of CI harboured *cfrB.* <3% of all isolates had neither *cfrA* or *cfrB, cfrB* more associated with CI than HI.This data analysis reveals potential gene targets towards vaccine development against *Campylobacter* strains.

**P483**

# How Pseudomonas aeruginosa utilises multiple Type VI Secretion systems and range of toxins to gain advantage in polymicrobial communities.

Marta Rudzite, Robert Endres, Alain Filloux
Imperial College London, London, United Kingdom

## Abstract

*Pseudomonas aeruginosa* is an opportunistic pathogen and its biofilm lifestyle is associated with chronic infections in people with pre-existing conditions. Establishment and survival of individual bacteria within dense polymicrobial communities is dependent on various factors including direct competition. A part of *P. aeruginosa* success in polymicrobial context can be attributed to its use of Type VI Secretion system (T6SS). T6SS is a bacterial macromolecular complex that can be used to inject toxins directly into adjacent prey cells. *P. aeruginosa* possesses 3 distinct T6SSs and a large number of varied T6SS effectors. T6SS toxins poses a diverse set of functions including disruption of cell wall integrity causing competitor lysis, degradation of nucleic acids and prey growth impairment via disruption of metabolism.

We generated a mutant collection lacking in individual toxin genes and T6SS machinery to investigate how *P. aeruginosa* gains a competitive edge in polymicrobial communities. By combining varied investigative approaches, we aim to understand how *P. aeruginosa* uses T6SS to shape dynamics of bacterial populations across systems of differing scale. By imaging whole mixed bacterial colonies, it is possible to investigate how action of individual T6SS toxins affects whole community structure. Subsequently, by incorporating single-cell data about T6SS firing behaviours, e.g. firing rate, and toxin modes of action within an agent-based theoretical modelling framework, we aim to connect single-cell behaviour with changes in whole population structure. This will allow to further understand how changes in individual bacteria firing strategy or toxins used lead to competitive advantages in the context of whole populations.

## P484

# Spatial pattern formation in bi-stable and coupled bacteria

Kevin Simpson[1], Juan Keymer[2,3,4], Fernán Federici[1,5,6]
[1]ANID – Millennium Science Initiative Program – Millennium Institute for Integrative Biology (iBio), Santiago, Chile. [2]Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile. [3]Departamento de Física, Pontificia Universidad Católica de Chile, Santiago, Chile. [4]Natural Sciences and Technology, Universidad de Aysén, Coyhaique, Chile. [5]Institute for Biological and Medical Engineering, Schools of Engineering, Medicine and Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile. [6]FONDAP Center for Genome Regulation. Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Santiago, Chile

## Abstract

Understanding how spatially-correlated cellular states emerge from local interaction of gene network dynamics is a fundamental challenge in biology. The study of gene spatial correlations emerging from cell-cell coupling in natural systems is difficult since complex interactions are the norm. An alternative is to generate synthetic genetic networks (SGNs) that capture essential features of cell-cell interactions and reveal their influence in the emergence of cellular patterns. Here, we combine synthetic biology, theoretical modelling and computational simulations to address possible mechanisms for multi-scale self-organization of gene states in bacteria. We constructed SGNs able to acquire two cellular states and interact locally by cell-to-cell signaling in *E. coli*. Inspired by the Ising model, we name these SGNs ferromagnetic or antiferromagnetic depending if they stabilize the same or the opposite state in neighboring cells. As predicted by our simulations that combine the Ising model with the Contact Process, the SGNs allowed the self-organization of short and long-scale spatial patterns of cellular state domains in colonies, where the size of the domains depends on the type of interaction. The emergence of spatial correlations showed to be independent of cell shape, and the autocorrelation function of ferromagnetic colonies follows a power-law with a critical exponent consistent with the Ising model at the critical transition. Deciphering the mechanisms that link SGN switching behavior to global distribution of states could shed light to the study of specialization and division of labor in bacterial communities, and provide guidance in the engineering of self-organized structures in spatially-arranged multicellular systems.

## P485

# Investigating the role of metal ions in the assembly of the *Caulobacter crescentus* S-layer

Matthew Herdman, Andriko von Kügelgen, Tanmay Bharat
University of Oxford, Oxford, United Kingdom

## Abstract

Surface layers (S-layers) are proteinaceous crystalline coats that constitute the outermost component of a majority of prokaryotic cell envelopes. S-layer proteins (SLPs) are a diverse class of molecules able to self-assemble into regular two-dimensional lattices, fulfilling a variety of biological functions, including evading predation, resisting environmental stress, and maintaining cell morphology. SLPs of multiple species have been shown to oligomerise in a metal-dependent manner, suggesting a potential universal principle that underpins S-layer biology. We have applied a variety of high-resolution structural and cell biology techniques to investigate the roles of metal ions in the formation of the *Caulobacter crescentus* monomolecular S-layer, comprised of the single bipartite SLP, RsaA. Through optical microscopy of fluorescently tagged S-layers, we show that calcium ions are an essential component of S-layer crystallisation and attachment to the cell surface. Additionally, through electron cryomicroscopy structure determination in conjunction with long-wavelength X-ray anomalous diffraction experiments, we have mapped the positions of metal ions in the S-layer at near-atomic resolution. These experiments address unanswered questions regarding the binding properties of RsaA's canonical metal binding sites and confirm our predictions from cellular data. These findings contribute to our understanding of how *C. crescentus* cells are able to form a regularly arranged S-layer on their surface and have implications in fundamental prokaryotic biology related to S-layers, with potential impact into the synthetic biology of biomaterials using SLPs.

**P486**

# Over expression of regulation of genes for peptide formation to revolutionization of evolution in microorganisms .

Misbah Jamil
University of the punjab, Lahore, Pakistan

## Abstract

Bacterial roles or consideration relation for different purposes varies with different destination depends on innovation goals . some historical purposes regarding with human demands open many new foldes towards different direction in the field of microbial biotechnology . In this regard advances in microbial technological purposes was taken into consideration with specific emphasis on microorganism as a model of studies . As gram positive bacteria especially the bacillacea constitute the ability to convert naturally product to chemical formation of peptide expression . so its introduce possibility to employ more rational diversion to strains modification because microrganism consist of gene encoding enzymes that regulate this protraction as a modulators. For this purposes desired strains cfu was isolated by employing general method of microbiology . For further stability of experiment was recuperate for the enhancement of shelf life of microorganism to revolutionization of evolutionary purposes for genetic manipulation . Expression of mutagenic attributes was consider as basic inflicts to induce genetic mutation by phenotypic mutation as well as detectable alteration of genes . The objective of this studies directed towards more renovation of synergistic behavior of factors to confabulate more innovative pathways towards the protraction of peptide by employing this technology to resolve global use of microbial product to overcome economically issues

**P487**

# Evaluation of Drug Activity Against Staphylococcal Biofilms – Settings of Conditions Leading to Reproducible and Robust Biofilm Formation *in vitro*

Adéla Diepoltová, Klára Konečná, Ondřej Janďourek, Petr Nachtigal
Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Hradec Králové, Czech Republic

## Abstract

*Staphylococcus aureus* (SA) and *epidermidis* (SE) are the most common pathogens of the genus *Staphylococcus*, causing biofilm-associated infections. Bacteria in biofilms are difficult to eradicate due to their resistance and serve as a reservoir for recurring persistent infections. A variety of protocols for *in vitro* drug activity testing against biofilms has been introduced. However, there are often fundamental differences. In our pilot study, we developed optimal conditions for staphylococcal biofilm formation on plastic pegs in order to set a methodology for an evaluation of the antibiofilm activity of candidate molecules. The convenience of the plastic pegs lies in their removability from the lid for easy access to multiple equivalent biofilms, and in possibility of *in situ* detection and quantification by confocal laser microscopy. For the purpose of enhancement in staphylococcal biofilm formation, the impact of peg surface modification with 3 different coating materials was studied as well. An increase of biofilm biomass was evaluated by crystal violet staining method. The basic precondition for obtaining relevant and reproducible data regarding antibiofilm activity is the formation of robust biofilms with typical attributes such as the presence of a biofilm matrix. In our study, *in vitro* conditions revealed that we fully met the preconditions for the SA and methicillin-resistant SA strains. In conclusion, we demonstrated statistically significant enhancement of biofilm formation in all studied staphylococcal strains, including either strong biofilm producer phenotype (SA, methicillin-resistant SA) and weak biofilm producer phenotype (SE).

Supported by the SVV Project No. 260549 and by the Czech Science Foundation project No. 20-19638Y.

**P489**

# How do bacteria change their coats? Structural analysis of acyltransferases involved in O-antigen modification

Sarah Tindall, Marjan van der Woude, Gavin Thomas
University of York, York, United Kingdom

## Abstract

Acyltransferase-3 (AT3) domain-containing proteins are involved in acylation of a diverse range of carbohydrates across all domains of life. In bacteria they are essential in processes including symbiosis, antimicrobial resistance, and antibiotic biosynthesis. Despite this, the mechanism of action is largely unknown. AT3 proteins from Salmonella spp. are responsible for acetylation of lipopolysaccharides which can generate a specific immune response upon infection. Here we analysed two AT3 proteins from Salmonella spp., some differences exist but both contain an integral membrane AT3 domain fused to a periplasmic SGNH domain. Identification of essential residues from each domain suggests both domains are required for acylation.

The crystal structure of the SGNH domain and periplasmic linking region was determined. Novel structural features are seen in comparison to other SGNH domains. In particular, the periplasmic linking region is structured and forms an extension of the SGNH domain (SGNH-extension). Removal of the SGNH-extension suggests that this region is important for stability of the SGNH domain. The structure of the SGNH-extension suggests the SGNH domain is in close proximity to the acyltransferase domain and the domains may interact. In silico co-evolution analysis, used to make predictions about the structure of both domains, suggests likely inter-domain interactions. This analysis also predicted which transmembrane helices in the acyltransferase domain interact giving an insight into the overall structure.

Combining these data we propose a refined model of AT3-SGNH proteins which enhances our understanding of the mechanism and function of AT3 proteins required for modification of cell-surface carbohydrates.

## P491

# Bioinformatic insights into the stability systems of *Yersinia ruckeri* plasmid pYR4

Luke Hamstead1, Jack Leo, Gareth McVicker
Nottingham Trent University, Nottingham, United Kingdom

## Abstract

*Yersinia ruckeri* causes enteric redmouth disease in salmonids fishes, which presents an economic threat to fish farms. Plasmids are commonly associated with *Y. ruckeri* isolates. *Yesinia ruckeri* NVH_3758 was isolated in 1987 and its genome sequenced in 2017. The genome assembly includes an ~81kb plasmid (pYR4). Previous work shows that pYR4 shares a 55kb locally collinear block (synteny block), with >99% nucleotide identity, with pYR3. Toxin-antitoxin (TA) systems maintain plasmids through post-segregational killing of daughter cells that do not inherit the plasmid. Bioinformatics tools were used to predict TA systems and explore the phylogeny of this plasmid. The toxins were compared with known tertiary structures to model the toxins and their likely mechanisms of action. Bioinformatic analysis revealed one complete *higBA* TA operon. A further incomplete TA system reveals phylogenetic links with a complete TA operon on *Serratia fonticola* pSF001 and pSF002 and *Pectobacterium parmentieri* pPAR01. These plasmids share 23%, 19%, and 12% query cover, respectively. They include a complete TA system that is incomplete in pYR4, but not the *higBA* operon. HigB is homologous with the tertiary structure and inhibition mechanism of a *Vibrio cholerae* chromosomal toxin. Operons and synteny blocks were run through NCBI BLAST; the whole plasmids were then aligned with pYR4. A flocking algorithm was used to display a horizontal gene transfer (HGT) network from plasmids with identified synteny with pYR4. This research shows the importance of horizontal gene co-transfer of TA systems and virulence factors for virulence maintenance.

**P492**

# New insights into the quorum sensing PqsE-mediated regulation of the *pqsA* promoter in *Pseudomonas aeruginosa.*

Natalia Romo[1], Giordano Rampioni[2], Stephan Heeb[1,3], Miguel Cámara[1,3]
[1]School of Life Sciences, Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom. [2]Department of Science, University Roma Tre, Rome, Italy. [3]National Biofilms Innovation Centre, Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom

## Abstract

The *Pseudomonas aeruginosa pqs* quorum sensing system controls the expression of many virulence traits using the Pseudomonas quinolone signal (PQS) synthesized by the *pqsABCDE* operon, which expression is mainly activated by PqsR upon binding to PQS. PqsE, in addition to having a role in the biosynthesis of PQS, is also the main effector of the *pqs* system through a still obscure mechanism. Among others, PqsE positively regulates genes involved in the biosynthesis of pyocyanin, elastase, and rhamnolipids as well as genes related to biofilm formation. Furthermore, it balances the production of PQS by repressing the *pqsA* promoter, however, since PqsE does not possess a DNA-binding domain, the molecular mechanism underlying this repression remains elusive. Using a DNA pull-down analysis to identify proteins that bind to the *pqsA* promoter upon *pqsE* overexpression we found the hypothetical protein PA27NR and the denitrification regulatory protein NirQ. Moreover, using deletion, site-directed mutagenesis, as well as transcriptional and translational reporter assays we show that: (i) albeit not essential for the action of PqsE, both PA27NR and NirQ play a role in the regulation of the *pqsABCDE* operon; (ii) the PqsE-mediated repression towards the *pqsA* promoter is at the post-transcriptional level and, (iii) PqsR is indispensable for this repression. These results provide new insights into the regulatory relationships between PqsE and the *pqs* operon and further demonstrate the complex dynamics of the regulation of the *P. aeruginosa pqs* system.

**P493**

# How can ammonia-oxidizing archaea (AOA) cope with oxygen limitation? Insights into their ecophysiology at nanomolar oxygen concentrations.

Alejandra-Elisa Hernandez-Magaña, Beate Kraft
Nordcee, Biology Department, University of Southern Denmark, Odense, Denmark

## Abstract

Ammonia-oxidizing archaea (AOA) use oxygen to oxidize ammonia to nitrite, playing a major role in nitrification. Despite their dependence on oxygen, AOA are widely distributed and abundant in nature, even in environments with very low oxygen concentrations, such as Oxygen Minimum Zones (OMZ) in the ocean or marine anoxic/hypoxic sediments.

Their abundance in such oxygen-depleted environments suggests that some ammonia oxidizers are capable of coping with oxygen limitation but is still unknown what this mechanism would be and what the implications are for their ecology and distribution. In order to resolve the role and activity of AOA in oxygen-limited environments, the physiology of different pure isolates was explored through experiments under oxygen-limitation (nanomolar range).

Oxygen and nitric oxide (NO) were measured with ultra-trace-range (nanomolar range) oxygen optodes and NO microsensors, respectively. Molecular nitrogen ($N_2$) and nitrous oxide ($N_2O$) was measured through $^{15}N$ stable isotope labelling and Isotope Ratio Mass Spectrometry. Our results show ongoing ammonia-oxidation even under oxygen depletion. These novel insights are important to understand how some ammonia oxidizers can survive and be abundant in oxygen-limited ecosystems. Moreover, these observations are relevant for the better understanding of $N_2O$ and NO production by ammonia oxidizers when exposed to oxygen-limitation.

**P495**

# Investigating the metabolic ability of *Campylobacter jejuni* using an integrated *in silico* and *in vitro* approach

Claire Hill, Noemi Tejera, Dipali Singh, John Wain
Quadram Institute Bioscience, Norwich, United Kingdom

## Abstract

Campylobacter are the most common cause of human bacterial gastroenteritis worldwide. The most frequently reported in human diseases are *C. jejuni* and *C. coli*. In the UK, over 80% of human gastroenteritis is caused by *C. jejuni* and around 10% to *C. coli*. Symptoms of campylobacteriosis such as diarrhoea, fever, nausea last between 2 to 5 days. Antimicrobial treatment is only recommended in invasive cases but this proportion has increased recently making Campylobacter a serious and growing public health threat and a priority to for novel control strategies.

In this study, we have combined an *in silico*, metabolic modelling technique and an *in vitro* growth experiment with biochemical analysis to understand and explore the metabolic capabilities of *C. jejuni* and link between genotype and phenotype. We have constructed and curated a genome-scale metabolic model for *C. jejuni* M1cam, validated it for energy and mass conservation and experimentally observed the proportion of biomass components produced. The model is used to identify cellular functions or metabolic routes that are crucial for growth and/or survival, using Flux Balance Analysis. In this work, we particularly focus on flexibility in energy metabolism of Campylobacter, through both oxidative phosphorylation and substrate-level phosphorylation, its ability to use a wide variety of substrates as electron donors and acceptors and the role of oxygen and hydrogen on the electron transport chain. Understanding the fundamental basis of how Campylobacter utilises the resources available in it's environment is crucial for combatting the emerging threat to public health.

## P496

# A potential new paradigm of denitrification in Neisseria gonorrhoeae

Jack Bolton, Karrera Djoko
University of Durham, Durham, United Kingdom

## Abstract

Metals are bacterial nutrients. Upon infection by microorganisms, the animal host innate immune system typically reduces the availability of metals. In response, bacterial pathogens can activate pathways for metal uptake to avoid metal starvation. This competition for metals at the host-pathogen interface is termed "nutritional immunity".

We are interested in how the obligate human pathogen *Neisseria gonorrhoeae* acquires nutrient copper (Cu). This Gram-negative bacterium expresses several respiratory cuproenzymes that are required for growth and metabolism in both aerobic and anaerobic conditions. These cuproenzymes include the putative nitrous oxide reductase (NosZ). NosZ contains 12 Cu atoms per functional dimer and it catalyses the reduction of nitrous oxide ($N_2O$) to dinitrogen ($N_2$). This reduction is an intermediate step in the denitrification pathway, in which nitrite ($NO_2^-$) is used as the terminal electron acceptor for respiration instead of $O_2$. In this project, we will determine whether NosZ is expressed as a functional enzyme in *N. gonorrhoeae*. We will then examine how this enzyme acquires nutrient Cu and subsequently assembles its active site.

Given the rise in antibiotic resistance and the worldwide recognition of multidrug-resistant *N. gonorrhoeae* as a major threat to public health, we hope that a fundamental understanding of the physiology and metabolism of this organism will yield new strategies for anti-infectives, for instance by manipulating Cu availability at the site of infection.

**P497**

# Regulatory mutants of *Serratia* with modulated antibiotic production due to intergenic transposon insertions

Daniel Plaza, George Salmond
University of Cambridge, Cambridge, United Kingdom

## Abstract

Antibiotic resistance is an increasing problem, exacerbated by global dissemination of drug resistance genes under selection pressure. Moreover, the rate of new antibiotic discovery declined over previous decades and so there is a growing need for new antibiotic discovery and a deeper appreciation of the various genetic and physiological factors that influence antibiotic biosynthesis.

The enterobacterium *Serratia* sp. ATCC 39006 (*Serratia*) is a useful model for studies on the biosynthesis and regulation of bioactive secondary metabolites, including two antibiotics - a carbapenem and prodigiosin. Both compounds are tightly regulated in response to various physiological and environmental signals, including quorum sensing.

We have identified novel regulators of antibiotic production after random transposon mutagenesis. Multiple regulatory mutations mapped to a small locus defining an intergenic region (IGR). These IGR insertion mutants display elevated production of both the carbapenem and prodigiosin antibiotics and they also exhibit reduced motility, confirming pleiotropic impacts. Exploiting phage transduction, we constructed double mutants with 15 known *Serratia* regulators and showed that the IGR mutant phenotype was epistatic over several of these. Further analysis suggested the presence of a putative gene encoded within the IGR locus, which may play an impactful role in the intricate regulatory network. Functional characterisation of the IGR region and its physiological impacts in both the modulation of antibiotic production and in wider pleiotropy were dissected. Mechanistic understanding of the regulatory mechanisms involved may prove exploitable in enhancing controlled antibiotic hyperproduction.

## P498

# Utilization of CRISPR interference to knock down bricks in the mycobacterial cell wall

Bala Tripura Sundari Annapurna Madduri, Luke Alderwick
University of Birmingham, Birmingham, United Kingdom

## Abstract

*Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis continues to bewilder the scientific community with the emergence of multidrug and extensively drug resistant strains. *Mtb* survives and persists in toxic bactericidal milieu within host immune cells owing to its highly impermeable cell envelope that shields the *Mtb* bacilli. In this regard, the mycolyl-arabinogalactan-peptidoglycan (mAGP) complex sits at the core of the cell envelope, allowing for further molecular decoration with virulence factors and immunomodulators that collectively contribute to the virulence and pathogenesis of *Mtb*. Among the numerous enzymes involved in mycobacterial cell wall biosynthesis, arabinofuranosyltransferases (Aft's) are involved in the biosynthesis of arabinan domain of the mAGP complex. Knocking down a putative essential gene AftC, results in the generation of a truncated, immunostimulatory, Toll-like receptor 2 (TLR2) antigen – Lipomannan (LM) instead of Lipoarabinomannan (LAM) within its cell envelop. We have generated AftC – knock downs in *Mycobacterium bovis* BCG, a surrogate organism to study *Mycobacterium tuberculosis* using the novel CRISPR interference technique with a significant growth defect, and phenotypic alterations. We have characterised the effect of AftC knock down in planktonic and biofilm cultures of *M. bovis* BCG, as well as its infectivity and immunostimulatory properties in THP1 human macrophage cell line.

**P499**

# Biochemical and Biophysical Characterization of Exoribonucleases from the Human Pathogen *Mycobacterium tuberculosis*

Vanessa Costa, Margarida Archer, Cecília Arraiano, José Brito, Rute Matos
Instituto de Tecnologia Química e Biológica António Xavier, Lisbon, Portugal

## Abstract

*Mycobacterium tuberculosis* remains the leading cause of mortality from a single infectious organism, infecting nearly one-third of the global population. The current emergence of multidrug-resistant strains represents a serious health problem nowadays. Moreover, regulation of gene expression through RNA metabolism is a key mechanism for bacterial growth, division and rapid accommodation to environmental conditions. Ribonucleases are enzymes present in all living organisms that play an important role in RNA processing and degradation. Particularly, ribonucleases belonging to the RNB-family are often essential for viability of prokaryotes and are implicated in the establishment of virulence of several pathogens. In the present study we aim to structurally and functionally characterize two putative exoribonucleases from the RNB-family of enzymes in *M. tuberculosis*. Overexpression and purification of the proteins was performed and further *in vitro* activity, binding and helicase assays using synthetic RNA substrates were accomplished. In parallel, a biophysical characterization proceeded with several crystallization trials and protein stability tests. We have demonstrated that both RNases are 3'-5' exoribonucleases with different degradation properties and unravelled the importance of highly conserved residues for catalysis. Moreover, we were able to identify improved buffer formulations that increase protein stability, possibly enhancing their propensity to crystallize. The information regarding RNA metabolism in *M. tuberculosis* is limited and RNB-family enzymes have not been previously characterized in this important human pathogen. Thus, a complete knowledge of these ribonucleases is an approach to recognize their influence in *M. tuberculosis* metabolism and to better understand the post-transcriptional control in this pathogen.

## P500

# Cell-to-cell ATP differences can modulate cellular decision-making

Ryan Kerr[1], Sara Jabbari[1], Iain Johnston[2]
[1]University of Birmingham, Birmingham, United Kingdom. [2]University of Bergen, Bergen, Norway

## Abstract

Cells generate phenotypic diversity both during development and in response to stressful and changing environments, aiding survival. Functionally vital cell fate decisions from a range of phenotypic choices are made by regulatory networks, the dynamics of which rely on gene expression and hence depend on the cellular energy budget (and particularly ATP levels). However, despite pronounced cell-to-cell ATP differences observed across biological systems, the influence of energy availability on regulatory network dynamics is often overlooked as a cellular decision-making modulator, limiting our knowledge of how energy budgets affect cell behaviour. Here, we consider a mathematical model of a highly generalisable, ATP-dependent, decision-making regulatory network, and show that cell-to-cell ATP variability changes the sets of decisions a cell can make. Our model shows that increasing intracellular energy levels can increase the number of supported stable phenotypes, corresponding to increased decision-making capacity. Model cells with sub-threshold intracellular energy are limited to a singular phenotype, forcing the adoption of a specific cell fate. We suggest that energetic differences between cells may be an important consideration to help explain observed variability in cellular decision-making across a broad range of biological systems, including bacteria and the blood stem cell system.

*NOTE: Our work is highly interdisciplinary and if you believe it would be better suited to another topic area, then I would be delighted to discuss that further.

## P501

# Uncovering the residues responsible for Dis3L2 specificity

Susana M. Costa, Rute G. Matos, Sandra C. Viegas, Cecília M. Arraiano
Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

## Abstract

Exoribonucleases from the RNB-family of enzymes are widely distributed in nature. DIS3 is the eukaryotic homolog of the bacterial exoribonuclease II and is the only catalytic subunit of the core exosome complex. In humans, there are three members of DIS3 family that can be distinguished according to the sequence conservation of the active site: DIS3, DIS3L (DIS3L1) and DIS3L2. Unlike its family counterparts, DIS3L2 does not interact with the exosome since it lacks the PIN domain, which is essential for the interaction with this multiprotein complex. Dis3L2 is involved in several cellular mechanisms, such as apoptosis, cellular differentiation and proliferation and its mutations have been associated with Wilms tumor formation and Perlman syndrome in children. Distinct studies on Dis3L2 enzyme unraveled a novel eukaryotic RNA decay pathway that challenged the models already established. Dis3L2 activity is stimulated by the addition of untemplated uridine residues to mRNAs, tRNAs, microRNAs, snRNAs among other classes of RNA.

The first insight on the uridylation involvement in controlling the stability of poly(A)-containing mRNAs was reported in *S. pombe*. However, the precise mechanism of action of this enzyme is not yet fully understood. In this work, the activity of fission yeast Dis3L2 mutant proteins was analyzed over different RNA substrates. The aim was to characterize the amino acid residues that distinguish Dis3L2 substrate specificities regarding its family homologues, namely the preference for uracil residues. Moreover, this will enable us to understand its mechanism of action and its function in different eukaryotic cells.

## P502

# Characterisation of minimal metabolic networks by complex network theory reveals new functional class of genes.

Giorgio Jansen[1], Grigoris Amoutzias[2], Vito Latora[3], Giuseppe Nicosia[4], Stephen Oliver[1]
[1]University of Cambridge, Cambridge, United Kingdom. [2]University of Thessaly, Larissa, Greece. [3]Queen Mary University of London, London, United Kingdom. [4]UNiversity of Catania, Catania, Italy

### Abstract

Construction of minimal metabolic networks would contribute both to our understanding of the origins of metabolism and to the efficiency of biotechnological processes by reducing the opportunities to divert flux away from desired products. We have designed minimised metabolic networks using a novel *in silico* synthetic biology pipeline that removes genes encoding enzymes and transporters from genome-scale metabolic models. The resulting minimal gene-set still ensures both viability and a growth rate value close to that of wild type.

The composition of these *MMNs* has defined a new functional class of genes that we term *mandatory.* These genes, whilst not essential, are very rarely eliminated in the construction of an *MMN,* suggesting that it is difficult for metabolism to be re-routed to obviate the need for such genes. We have characterised the place of these genes in the genetic interactions network using complex network theory. The average degree distributions of *MMNs* for aerobic and anaerobic growth are distinct, but are similar for media with different chemical compositions. Moreover, *MMNs* with a higher number of mandatory genes removed show a significantly reduced efficiency.

Further analysis of the mandatory genes, using both network and bioinformatic approaches has revealed that not only do these genes have more genetic interactions than the bulk of metabolic genes but their protein products also show more protein-protein interactions. In yeast, mandatory genes are predominantly single-copy and are highly conserved across evolutionarily distant organisms. These features may explain why mandatory genes are so difficult to remove from the metabolic network.

## P503

# The RNA chaperone Hfq is involved in the oxidative stress response of *Listeria monocytogenes*

André Seixas, Cecília Maria Arraiano, José Marques Andrade
ITQB NOVA – Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

## Abstract

The RNA chaperone Hfq is an important bacterial post-transcriptional regulator. In Gram-negative bacteria its main role is in riboregulation, promoting the interaction between non-coding sRNA and mRNA, a function that was also associated with stress response in *E. coli* and *Salmonella*. However, in Gram-positive bacteria like *Listeria monocytogenes*, Hfq main role remains elusive as this protein seems to be expendable for riboregulation.

Here we found that Hfq is essential for the oxidative stress response of *Listeria*, with disruption of the hfq gene resulting in a hypersensitive phenotype to hydrogen peroxide ($H_2O_2$). Using a sub-inhibitory concentration of $H_2O_2$, the growth of the wild-type strain was barely affected and recovered immediately upon addition of the oxidative stressor. However, the hfq null-mutant culture could not resume growth. This growth inhibition after stress was further confirmed in solid medium, with the hfq mutant showing reduced viability upon exposure to $H_2O_2$ when compared to the wild-type strain. $H_2O_2$ is one of the several reactive oxygen species (ROS) found inside cells, and $H_2O_2$ decomposition is mainly mediated by catalase. An enzymatic activity assay demonstrated that catalase is less active in the absence of Hfq when compared to the wild type. We are currently analysing how Hfq may affect catalase activity and/or expression.

The results obtained here associates, for the first time, Hfq and the way *Listeria* copes with oxidative stress. This may contribute to understand novel regulatory pathways controlling the oxidative stress response of an intracellular pathogen.

**P504**

# DraG: a novel pleiotropic regulator of gas vesicle and secondary metabolite biosynthesis in *Serratia* sp. ATCC 39006

Carlo Miguel Sandoval, George Salmond
University of Cambridge, Cambridge, United Kingdom

## Abstract

*Serratia* sp. ATCC 39006 (S39006) is a rod-shaped, motile, Gram-negative bacterium that produces a $\beta$-lactam antibiotic (a carbapenem) and a bioactive tripyrrole red-pigmented antibiotic, prodigiosin. It is also the only known enterobacterium which produces gas vesicles (GV) naturally, enabling flotation. The regulation and biogenesis of GVs is a complex process and is part of overlapping regulatory networks affecting various phenotypes, including the biosynthesis of the carbapenem and prodigiosin.

In our search for novel inputs to the complex GV network, we generated a random transposon mutant library and isolated strains with defective GV biosynthesis phenotypes. One mutant had a transposon insertion in the dinitrogenase reductase-activating glycohydrolase (*draG*) gene. The *draG* mutant had perturbed GV biosynthesis and flotation, reduced prodigiosin and cellulase production, but showed increased swimming and swarming motilities. Notably, carbapenem antibiotic production was abolished in the *draG* mutant. The observed pleiotropic impacts indicate that *draG* is a positive regulator of cellulase, prodigiosin and carbapenem production and a repressor of motility in S39006, which we have confirmed through comparative quantitative analysis of the mutant's intracellular proteome against the wild type. These results suggest *draG* is a novel pleiotropic co-regulator of GV morphogenesis and bioactive secondary metabolite production in S39006.

## P506

# Development of a genome-scale metabolic model for Avian Pathogenic Escherichia coli (APEC)

huijun long, Arnoud H. M. van Vliet, Huihai Wu, Roberto M. La Ragione, Jai Mehat
university of surrey, surrey, United Kingdom

## Abstract

**Background:** Avian Pathogenic *Escherichia coli* (APEC) is primarily associated with extra-intestinal infections in birds and is a significant economic issue. Therefore, a greater understanding of the biology of APEC is required to assist in the development of interventions. Here we present a metabolic model for APEC to assist in the identification of targets for the generation of live-attenuated vaccines or the development of novel anti-APEC antimicrobials.

**Methods:** n=114 APEC isolates were genome sequenced and the sequences uploaded to RAST and linked to biochemical reactions encoded by the Model SEED. Gap filling was performed by CROBA, *E. coli* *i*JO1366 was used as reference model. The metabolic model was validated using BioLog phenotype microarrays (PMs) and three strains chosen from phylogroups B2, G and C.

The *lysA* gene encodes the final enzyme in the lysine biosynthesis pathway and was predicted as essential gene by our APEC model. Here the entire *lysA* gene was knocked out by deleting and replacing it with a chloramphenicol resistance gene (cat) for model validation and to investigate the effect of lysine auxotrophic phenotype on growth phenotype.

**Results:** 1848 metabolic reactions were predicted. The correlation coefficient between model predicted growth and PMs data of chosen APEC strains were 0.64 (C), 0.64 (G) and 0.55 (B2), respectively which indicated a better agreement with our APEC collection compared to *iJO1366* model under the same media conditions. The model accurately predicted lysine as an essential pathway for APEC and a *lysA* mutant was observed to be auxotroph for lysine.

## P507

# Insights into Otitis Media: Dissecting the interaction of C-Reactive Protein with Non-Typeable Haemophilus influenzae

Gurpreet Bharj[1,2], Pratik Vikhe[1], Steve Brown[1], Derek Hood[1]
[1]MRC Harwell, Oxford, United Kingdom. [2]University of Oxford, Oxford, United Kingdom

## Abstract

Otitis Media (OM) is the inflammation of the middle ear (ME). Non-typeable Haemophilus influenzae (NTHi) is one of the leading otopathogens in causing OM. Phosphocholine (PCho) on the NTHi lipopolysaccharide influences host-pathogen interaction. C-Reactive Protein (CRP), an acute phase protein recognizes PCho, and can mediate bacterial killing. However, some strains of NTHi survive even in the presence of CRP. We aim to study the interaction of CRP with NTHi to understand its role in bacterial survival and OM.

NTHi can efficiently infect the *Junbo* mouse, a characterised model of chronic and acute OM. CRP levels were highest 1 day post-intranasal inoculation in the ME fluid (MEF) and nasal passage (NP) washes. We show CRP is a localized response to NTHi as serum CRP levels were unaffected in NTHi inoculated and non-inoculated mice at 1, 3 and 7-day post intranasal inoculation. Further, we confirm the presence of NTHi influences CRP levels in the MEF and NP washes. We show CRP binding is influenced by the position and expression of PCho on the NTHi surface. Serum bactericidal assays indicate that the expression and position of PCho affects NTHi survival. The removal of CRP from the serum restores NTHi survival. The expression of PCho also influences opsonophagocytosis activity in macrophages, thereby confirming the importance of PCho in NTHi survival.

The CRP-NTHi interaction is currently under investigation to advance our understanding of its role in the complex biological processes that influence bacterial killing and the onset, progression and resolution of OM caused by NTHi.

## P508

# Embedding 21st century employability into assessment and feedback practice through a student-staff partnership

Nicola Veitch, Anna Mcgregor, Fiona Stubbs, Miranda Broom, Dzachary Zainudden
University of Glasgow, Glasgow, United Kingdom

## Abstract

Although commonly thought to be only a measure of academic performance, assessments can also provide students with an opportunity to apply skills and knowledge acquired at university into written literacies that prepare them for the transition into prospective careers. Within a Level 2 Microbiology and Immunology course, students struggled to engage with standalone timetabled careers-related sessions, yet they showed enthusiasm when employability was embedded into assessments. A staff-student partnership project explored these issues, with the overall aim of understanding how to effectively embed employability skills into assessment and feedback, which supports the theme of supporting students to position themselves for the future.

Through student-run focus groups, this project investigated the reasons for low student engagement with the timetabled employability session and used student views to develop digital initiatives that could be applied to assessment and feedback practice with an emphasis on developing 21st-century competencies. These initiatives were then implemented as pre-session self-directed activities, which helped students link course feedback with employment skills and future career planning, followed by a newly developed in-class reflective feedback session that allowed students time to consider what skills they have developed and make links with future careers. Project evaluation was conducted using a quantitative survey of students involved.

This project aims to make feedback more meaningful and better valued by students. The approaches were designed in partnership with former students from the course, this project possesses the unique ability to deliver a genuine student voice both on employability concerns and the role of assessment.

## P509

# Teaching Microbial Genomics in the COVID-19 age: principles, theory, and practice

Jem Stach, Jon Marles-Wright, Maria del Carmen Montero-Calasanz
Newcastle University, Newcastle upon Tyne, United Kingdom

## Abstract

The importance of genomics in the COVID-19 age cannot be overstated and genomics has a key place in the advanced undergraduate microbiology curriculum. The COVID-19 pandemic, and its attendant lockdowns, have necessitated a change in the delivery of our microbial genomics module. The key challenges for delivering a remote computer-based genomics module, are ensuring student engagement and learning; and supporting the technical aspects of remote computer work.

In the absence of in-person classes, we have adapted our material covering the principles and theory of microbial genomics, to asynchronous videos and synchronous online workshops. Practical training for all undergraduate microbiologists in the UK has been severely reduced, which has forced us to focus skills training towards computational aspects of genome assembly, analysis, and interpretation. The set up and implementation of a robust and scalable Linux-based genome analysis pipeline for students presents many challenges: from organising remote access to computer clusters; software support; and managing hardware.

Assessment for the module is based on the demonstrating learning and skills development through the analysis of SARS-CoV2 genomes to identify spike protein variation and mapping this to published structures. Terminal assessment is through the analysis of newly sequenced bacterial genomes and the preparation of a genome report suitable for publication.

We will outline the implementation and management of our Microbial Genomics module as a model for computer-based skills training for undergraduate microbiologists. Furthermore, we will discuss the impact of the COVID-19 pandemic on the module and the opportunities it has presented.

## P510

# 'Drugs vs Bugs': raising awareness against antibiotic resistance through a boardgame

Célia Souque[1], Mathupanee Oonsivilai[2], Guillaume Souque[3], Ben Cooper[1]
[1]University of Oxford, Oxford, United Kingdom. [2]Mahidol-Oxford Research Unit, Bangkok, Thailand.
[3]Polytechnique Montréal, Montréal, Canada

### Abstract

Raising awareness is crucial to tackle the problem created by antibiotic resistance, but making such a complex topic relatable can be difficult. Game-based learning is a powerful tool to raise awareness as it allows the players to learn about a subject by experiencing directly the consequences of their actions.

We created an educational boardgame in Bangkok, Thailand, called 'Drug vs Bugs'. In this game, players take the role of doctors treating patients infected by viruses and bacteria. As the game goes on, players face increasing resistance in bacteria, linked to their collective use of antibiotics. The game mechanisms focus on transmitting three key messages: the deleterious effect of inappropriate antibiotic use, the importance of infection prevention through handwashing and vaccines, and the necessity to develop new treatments and diagnostic techniques. We created versions of the game both in English and Thai, as well as a simplified version for younger audience. We conducted outreach sessions thorough the development process in Thailand and in the UK, both in schools and in the community, reaching around 200 participants of various ages and backgrounds. Enjoyment, ease of play and perceived learning were measured through feedback questionnaires and most players found the game fun and engaging, and mentioned regularly the game key messages in their feedback.

The game is now available on a dedicated website as a free Print'n'Play' educational resource (https://drugs-vs-bugs.com/#/) and we are now working on a 'lesson pack' around the game to facilitate its implementation in the classroom.

## P511

# Improving the quality of the Intensive Care Unit (ICU) Microbiology ward round documentation through the introduction of a Microbiology ward round proforma

Pratap Harbham, Lucy Hamer, Ashraf Ariff, Mahesh Eddula, Balaji Ramamurthy
Sandwell and West Birmingham NHS Trust, Birmingham, United Kingdom

## Abstract

### Background

The GMC sets out clear standards expect from doctors when documenting in clinical notes. A failure to comply with these standards has both patient safety and medico-legal implications.

We aimed to audit the ICU Microbiology ward rounds documentation quality and improve inadequacies through a two-step quality improvement project.

### Methods

Baseline audit of ICU microbiology ward round documentation was conducted in October-2020 looking at documentation of i) current infection issues ii) current infection treatment iii) COVID results iv) Microbiology advice

Following this, a two-step intervention comprising an educational session, and the introduction of a microbiology ward round proforma was implemented. Re-audit was performed in December-2020.

### Results

Baseline data showed documentation generally was poor. Current infection issues and current infection treatment were documented in 64% and 72% of ward round entries respectively. Only 36% of entries documented COVID results. Microbiology ward round advice was present in 96% of entries.

Post-intervention, documentation of current infection issues and current infection treatment was much improved, present in 83% and 86% of entries respectively. Documentation of COVID results showed a statistically significant improvement, to 81% of entries, $t(19)=3.34, p=0.001691$. Microbiology ward round advice remained well documented at 90% of entries.

### Conclusion

Our study showed improvement in documentation quality through a quick and efficient intervention that is adaptable to both in person and virtual ward rounds. This can reduce the well-recognised risks of poor-quality documentation, including patient safety and medico-legal issues. Integrating the proforma template into electronic noting represents a future area to explore.


14–16 Meredith Street,
London, EC1R 0AB, UK
microbiologysociety.org