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POSTER ABSTRACT BOOK

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A001

Genomic Characterization of 2024 Dengue Outbreak in Federal District, Brazil

Lucas Luiz Vieira ORCID iD[1], Mariana Matos Roll ORCID iD[1], Brenno Henrique[1], Jordan Silva[1], Agenor Santos Junior[1], Alex Ranieri Jerônimo Lima ORCID iD[2], Gabriela Ribeiro[2], Maria Carolina Elias ORCID iD[2], Sandra Coccuzo Sampaio ORCID iD[2]

[1]Central Public Health Laboratory of the Federal District, Brasília, Brazil. [2]Center for Viral Surveillance and Serological Assessment (CeVIVAS), Butantan Institute, São Paulo, Brazil

Abstract

Dengue fever (DF) is a mosquito-borne endemic disease in South America and it is a major public health issue due to recurrent epidemic outbreaks. During the first half of 2024, the Federal District of Brazil (Midwestern region) experienced an increase of 1,119.5% in DF number of cases compared to the same period of 2023, reporting an incidence of 276,915 of probable cases (8,450.8 cases per 100,000 inhabitants). To understand the serotype and genetic diversity responsible for this outbreak, we conducted a genomic surveillance study of the circulating dengue viruses (DENV) in this city. A total of 47,013 serum samples were collected from DF-suspected patients originating from all regions of the Federal District. The diagnosis were confirmed by rRT-PCR assay in 55.3% (n= 25,995) of the cases. Serotyping revealed the presence of only two DENV serotypes. DENV-2 was the predominant serotype (88.8%; n= 23,087), followed by DENV-1 (11.2%; n= 2,908). Altogether 350 samples were submitted to whole-genome sequencing, and the phylogenetic analyses clustered the isolates into genotype V for DENV-1 and Cosmopolitan genotype for DENV-2, which are closely related to other Brazilian sequences, emphasizing the regional circulation of DENV. One of the major findings of this study was the predominance of the DENV-2 serotype in an outbreak in the Federal District of Brazil, which has never occurred since molecular surveillance was implemented. Moreover, these results may improve our understanding of the DENV transmission scenario and provide new insights into the co-circulation of different serotypes.

A002

Isolation and characterization of a novel lytic phage against drug resistant Acinetobacterbaumannii clinical isolates

Kareema Bahr[1], Ban Abdulsattar ORCID iD[2], Susan Ibrahim[3]

[1]College of science/Tikrit university, Tikrit, Iraq. [2]College of science/Mustansiriyah university, Baghdad, Iraq. [3]College of science/Mustansiriyah uonversity, Baghdad, Iraq

Abstract

Antibiotic-resistant *Acinetobacter*baumannii (A. baumannii) strains are increasing and emerging globally. A. baumannii has the ability to grow in the natural environment, especially near healthcare sites, with eradication difficulties. Bacteriophage is proposed as a safe alternative agent to control bacterial infection and contamination. A virulent bacteriophage named ΦKAB and its host bacterium were isolated from the Tigris River, Tikrit, Iraq, and characterized. The host and 60 clinical A. baumannii isolates were identified with 16SrRNA and bla_{OXA51}genes. Phage ΦKAB was classified as a member of the Myoviridae family in the order Caudovirales. The ΦKAB phage was capable of infecting 12 of the A. baumannii clinical isolates, with no lytic activity for other species tested. The phage showed a wide pH range tolerance (4-11) and thermal stability (-20°C- 50°C), with stability after preservation in different glycerol concentrations. In a single-step growth test, the phage showed a latent period of 10 min and a burst size of 104 virions/infected cell at an optimal multiplicity of infection of 10. Protein analysis of phage ΦKAB revealed 3 major bands and 3 minor bands with molecular weights ranging from 28 to 62 kDa. Biofilm production was detected by a microtiterplate assay, which showed that among 14 biofilm-producing isolates, including the host, 57% (8) were weak and 43% (6) were moderate biofilm producers. The phage anti-biofilm and synergistic effect of phage and colistin were tested. Therefore, the current study provides a new phage to the growing number of phages that specifically infect MDR A. baumannii.

A003

In Silico Assessment of Antiviral Potentials – Natural Compounds Against Nipah virus Precursor Glycoprotein

Sinethemba Yakobi ORCID iD

University of Fort Hare, Alice, South Africa

Abstract

The Nipah virus (NiV) is a highly pathogenic paramyxovirus that poses a significant threat to public health due to its high mortality rate and lack of effective therapeutics. This study investigates the potential antiviral activity of natural compounds—Quercetin, Resveratrol, and Berberine—targeting the F0 glycoprotein of the Nipah virus through molecular docking and molecular dynamics simulations. Using Schrödinger software for docking, followed by molecular dynamics simulations with Schrödinger, we assessed the binding affinities and stability of these compounds within the glycoprotein binding site. The MM/PBSA method was employed to calculate the binding free energies. Resveratrol emerged as the most promising compound with a highly favorable binding free energy of -43.15 kcal/mol, characterized by significant van der Waals interactions (-50.25 kcal/mol) and electrostatic contributions (-22.67 kcal/mol). The molecular dynamics simulations revealed stable binding interactions, supported by Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) analyses, which indicated minimal fluctuations and conformational stability of the Resveratrol-F0 glycoprotein complex. The computational findings were corroborated by detailed graphical representations of the interaction dynamics, providing insights into the rapid molecular events and conformational changes occurring during Resveratrol binding. Despite the desolvation penalty indicated by the polar solvation energy (35.50 kcal/mol), the overall binding free energy remained favorable, highlighting the strong affinity of Resveratrol for the F0 glycoprotein. This study underscores the importance of integrative computational techniques in antiviral drug discovery and supports the continued investigation of Resveratrol as a potential inhibitor of the Nipah virus.

A004

Role of genetic determinants in pathogenesis and evolution of chikungunya virus

GARVITA MATHUR, Sakshi Chaudhary, Shakuntala Mahilkar, Sujatha Sunil

ICGEB, Delhi, India

Abstract

Introduction- Chikungunya virus (CHIKV) causes major outbreaks every few years in India, with sporadic cases reported during the inter-epidemic period. Little is known about those genetic determinants that play decisive roles in an epidemic or an episodic outbreak of CHIKD.

Objectives- Generation and characterization of an infectious clone of Indian Chikungunya virus strain to understand the impact of genetic mutations found during the epidemic on CHIKV pathogenicity and its host.

Methods- Phylogenetic and whole genome sequence analysis was done to analyze circulating strains and unique mutations from clinical samples collected during epidemics in India. To understand these mutation impacts, an infectious CHIKV clone with the Indian strain was generated and characterized using plaque assay, replication kinetics, western blotting, and Immunofluorescence assay.

Findings- Our findings suggest that the ECSA genotype was circulating in India during the epidemic, and WGS analysis revealed five non-synonymous mutations correlated with severe clinical symptoms in patients. Characterization of infectious clones shows a similar pattern of replication kinetics with the highest peak at 24h and similar expression of E1 structural protein at 72h in Vero cells compared to the wild-type CHIKV, which suggests that the infectious clone-generated virus can be used as the platform to study different aspects of genetic mutations found during Indian epidemic.

Conclusion- The first infectious clone of CHIKV using the Indian strain has been generated to understand the role of genetic mutations on viral pathogenesis and host response.

A005

Single-cycle influenza viruses: a biosafe method for investigating highly-pathogenic H5N1 viruses

Sarah K. Walsh ORCID iD, Sarah J. Cole ORCID iD, Emma L. Davies ORCID iD, Matthew L. Turnbull ORCID iD, Edward Hutchinson ORCID iD

MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

The emergence of a panzootic clade of highly-pathogenic H5N1 (2.3.4.4b) in 2020, including its introduction into U.S. dairy cattle, has resulted in an increasing number of human exposures and spillover infections. While 2.3.4.4b H5N1 viruses remain highly virulent in birds and non-human mammals, their virulence in humans and the underlying molecular determinants of this virulence, remain unclear. Currently, research on highly-pathogenic influenza viruses is restricted due to the risk of inadvertently generating pathogens of pandemic potential. To mitigate this, we propose a biosafe experimental platform utilising single-cycle viruses which lack the haemagglutinin (HA) gene. The function of HA is provided in *trans* by a HA-expressing cell line, meaning that the single-cycle viruses are only capable of multi-cycle replication in the HA-expressing cell line, rendering them unable to infect humans or animals. Here, we present our work establishing this system using a single-cycle A/Puerto Rico/8/34 (PR8) virus. We created a HA-expressing MDCK cell line using lentivirus transduction, which achieved stable HA expression over 20 passages. The MDCK-HA cells were then used to rescue a ΔHA zsGreen-expressing single-cycle PR8 (ΔHA-PR8). ΔHA-PR8 could replicate in MDCK-HA cells but not in wildtype MDCK cells, confirming its single-cycle phenotype. Future work will ensure HA is not mobilised (*i.e.,* reacquired) by the single-cycle virus during serial passage before extending this approach to H5N1, pending biosafety approvals. This will allow us to investigate the cellular tropism, coinfection risk, and reassortment potential of H5N1 at reduced biocontainment levels, accelerating research on this important panzootic pathogen.

A006

Human astrovirus infection in pediatric patients with acute gastroenteritis in Thailand

Kattareeya Kumthip ORCID iD[1], Pattara Khamrin[1], Hiroshi Ushijima[2], Niwat Maneekarn[1]

[1]Chiang Mai University, Faculty of Medicine, Chiang Mai, Thailand. [2]Nihon University School of Medicine, Tokyo, Japan

Abstract

Human astrovirus is a common cause of acute gastroenteritis, particularly in young children worldwide. Investigating the molecular epidemiology of astrovirus is essential for monitoring the emergence of new strains and understanding the evolution of the virus circulating among children with acute gastroenteritis. This study aimed to examine the molecular epidemiology and genetic diversity of astrovirus in pediatric patients hospitalized with acute gastroenteritis in Chiang Mai, Thailand, from 2021 to 2023. A total of 1,124 fecal specimens were collected from children hospitalized with acute gastroenteritis during this period and tested for astrovirus infection using RT-PCR, followed by nucleotide sequencing and phylogenetic analysis. The overall prevalence of astrovirus infection was 2.0% (23/1,124). An increasing trend in astrovirus infection was observed after the COVID-19 pandemic, with annual incidence rates of 0.9%, 1.4%, and 3.4% in 2021, 2022, and 2023, respectively. Genotyping identified three major clades, classic human astrovirus (HAstV) and the newly emerging MLB and VA astroviruses, co-circulating in the study population. Five genotypes were detected, with HAstV1 as the most predominant (34.8%), followed by MLB1 and MLB2 (21.7% each), HAstV5 (13.1%), and VA2 (8.7%). These finding reveal the prevalence and diversity of astrovirus genotypes circulating among pediatric patients hospitalized with acute gastroenteritis in Chiang Mai, Thailand during 2021-2023, and provide valuable insights into the molecular epidemiology of astrovirus in pediatric patients with acute gastroenteritis.

A007

Evaluation of recombinant Infectious Bronchitis vaccine candidates against homologous challenge.

Sarah Keep ORCID iD, Katalin Foldes, Emily Briggs, Adam Rayment, Shafiyeel Chowdhury, Paul Britton, Erica Bickerton

The Pirbright Institute, Pirbright, United Kingdom

Abstract

The highly contagious *Gammacoronavirus* infectious bronchitis virus (IBV) results in significant economic losses to poultry industries worldwide. Live attenuated vaccines (LAVs) against IBV are generated by 80 – 100 serial passages of a field strain in embryonated eggs, balancing attenuation with the retention of immunogenicity. The molecular mechanisms underlying attenuation are unknown and vaccines present a risk of reversion to virulence. Over passaging can result in reduced vaccine efficacy. Vaccine induced immunity has been associated with the Spike (S) glycoprotein. The recombinant IBV (rIBV) BeauR-M41(S), in which the S gene of the attenuated rIBV Beau-R is replaced with the equivalent sequence from the virulent M41 strain, induces ~65% protection against M41 challenge. In an effort to increase protection to meet industry standards we generated two rIBVs based on BeauR-M41(S); 1) BeauR-Rep-M41-Struct in which all the structural, accessory, and 3' Untranslated region (UTR) sequence was replaced with that derived from M41 and 2) BeauR-M41(S)-L53S that contains a single point mutation (L53S) in non-structural protein (nsp) 16. *In vitro* assays demonstrated the L53S mutation enhances replication at biologically relevant temperatures. Chickens were vaccinated with either BeauR-M41(S)-L53S, BeauR-Rep-M41-Struct or BeauR-M41(S) and challenged 3 weeks later with M41. Vaccination with BeauR-Rep-M41-Struct did not significantly improve vaccine efficacy compared to BeauR-M41(S). BeauR-M41(S)-L53S did not induce any protective immunity despite stimulating antibody responses. Our study demonstrates the importance of the sequence accounting for the 5'UTR through to nsp 16 in the development of LAVs against IBV infection.

A008

Generation of fluorescently labelled spherical and filamentous influenza A viruses to study morphology and pathogenesis

Chidiebere F. Uchechukwu ORCID iD[1], Sammi Ta[1], Seema Jasim[2], Edward Roberts ORCID iD[2], Edward Hutchinson ORCID iD[2], Nicole C. Robb ORCID iD[1]

[1]University of Warwick, Coventry, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom

Abstract

Plasmid-based reverse genetics offers a powerful approach for generating recombinant influenza viruses with precise genetic modifications. In this study, we employed reverse genetic techniques to engineer fluorescently labelled spherical and filamentous variants of the influenza A virus. We successfully introduced tetracysteine motifs or ZsGreen fluorescent protein tags into the viral genome to generate recombinant viruses expressing fluorescent markers, while maintaining viral replication competence. The engineered viruses exhibited distinct morphological phenotypes, allowing for the visualisation and characterisation of viral morphology dynamics during infection. We aim to use live cell imaging techniques to investigate viral attachment, entry, and intracellular trafficking in host cells. Our findings will provide insights into the structural and functional differences between spherical and filamentous influenza A viruses, providing valuable information on virus-host interactions and pathogenesis.

A010

MOLECULAR DOCKING OF SARS-COV-2 SURFACE PROTEINS WITH SOME ACTIVE METABOLITES FROM PLANTS USED IN THE THERAPY OF COMMON COLD: POTENTIAL DRUG IDENTIFICATION.

Nkechi Egbe ORCID iD, Yahaya Umar, Uthman Shanono

Nigerian Defence Academy, Kaduna, Nigeria

Abstract

COVID-19 remains a global challenge, with current treatments and vaccines facing limitations such as resistance, limited accessibility, and emerging variants. Natural compounds offer a promising therapeutic avenue for addressing these issues, particularly in treating COVID-19 and other viral respiratory infections. Drugs derived from natural origin is considered as crucial therapeutic approach for the treatment of COVID-19 and other viral respiratory infections as such needs to be exploited for the treatment of the virus globally. The study aimed to computationally screen the phytochemicals of some Nigerian plants used in the therapy of common cold for anti-COVID-19 activity. Phytochemical analysis of the plant extracts was carried out using standard techniques, while bioactive compounds present in the extracts were detected by Gas Chromatography-Mass Spectrometry. The selected plant bioactive compounds were docked against the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp), Main protease (Mpro) and S protein-ACE2 targets, while Lopinavir, Remdesivir and Favipiravir were included as standard ligands. All the bioactive compounds exhibited acceptable drug-likeness and good oral bioavailability prediction, in addition to 89% of the compounds having slightly or practically non-oral toxicity using SwissADME and ProTox-II prediction servers. The overall result suggested that 3-Epimoretenol, Beta-Amyrin acetate, Methyl 3-oxours-12-en-23-oate, 20(29)-Lupenol acetate and Lanosterol acetate are the top most promising therapeutic bioactive natural compounds against SARS-CoV-2 Mpro, RdRp and spike protein when compared to the standard drugs. Taken together, data obtained indicate that these bioactive natural compounds may have a very good potential as anti-COVID-19 therapy.

A011

Characterisation of the antiviral properties of natural product MM 46115

Afifah Tasnim, Lona Alkhalaf, Greg Challis, Nicole Robb

University of Warwick, Coventry, United Kingdom

Abstract

The influenza virus is a major cause of respiratory tract infection, which results in 650,000 annual deaths worldwide. Although patients hospitalised with severe infection are given antiviral treatments, the virus has developed resistant mutations against many existing antivirals. Therefore, there is a crucial need for new, effective treatments.

We aimed to investigate the antiviral activity of a natural product called MM 46115 against the influenza virus. This spirotetronate polyketide is produced by the soil bacterial species *Actinomadura pelletieri* and has previously been demonstrated to have antiviral activity against several respiratory viruses.

In this study, plaque assays with MM 46115 showed a dose-dependent reduction (IC_{50} of 0.1235 µg) in viral titres against the influenza A/WSN/33 and B/Beijing/1/87 viruses. Cell viability and proliferation assays were also performed using Madin-Darby canine kidney (MDCK) cells and human lung adenocarcinoma (A549) cells which showed MM 46115 possessed a cytotoxic concentration of 123.5 µg. Therefore, it is possible to achieve anti-influenza activity with MM 46115 at non-toxic concentrations. Further plaque assays from infections performed over 24 hours showed treatment with MM 46115 produced lower viral titres for up to 6 hours, compared to without treatment. Although MM 46115 did not impair viral haemagglutination or neuraminidase activity, pre-incubation and post-infection assays showed MM 46115 is active within the first 2 hours of the virus life cycle.

Given the need for new therapies to deal with antiviral resistance, this study indicates MM 46115 and potentially other related compounds may be a promising alternative in response to resistant influenza.

A012

Toward the development of an assay to differentiate infected and vaccinated animals for classical swine fever C-strain vaccine

Sophie Harding ORCID iD[1], Amin Asfor[2], Helen Crooke[2]

[1]APHA, Weybridge, United Kingdom. [2]Animal and Plant Health Agency (APHA), Weybridge, United Kingdom

Abstract

Classical swine fever (CSF) is a notifiable viral disease of swine. The C-strain vaccine for CSF is highly effective and rapidly provides lifelong protection but is currently incompatible with differentiation of infected and vaccinated animals (DIVA). This project aims to develop a serological DIVA assay that can distinguish C-strain vaccinated and infected animals.

Monoclonal antibodies WH306 and WH310 have differential binding for field and vaccine strains of CSFV. These mAbs bind to an area in the D/A domain of the E2 protein in field strains but not C-strain. In-house competitive ELISAs were developed using E2 protein, adapted commercial kits or a truncated E2 protein comprising the D/A domain and were assessed for the differential detection of CSFV infection using these mAbs and sera from CSF-infected, vaccinated and naïve animals.

Sera from infected pigs competed for binding with WH306 in the adapted commercial ELISAs indicating that the epitope for this mAb is antigenic in swine. However, this test and the full-length E2 ELISAs were unsuccessful in differentiating sera from infected and vaccinated animals, although both could be separated from naïve animals. In contrast, the D/A assay differentiated between infected, vaccinated and naïve sera from a range of genotypes, but sera from vaccinated animals had higher than desired background.

The D/A ELISA was the most promising assay and will be evaluated further. Additional work is ongoing to identify peptides that can act as minimal epitopes in a similar competitive ELISA format through selective design and phage display.

A014

Picking Apart the Mechanism of Coronavirus Particle Assembly and Release

Conor Haydon[1], Isobel Webb ORCID iD[2], Sarah Keep ORCID iD[1], Andrew D Davidson ORCID iD[2], Erica Bickerton ORCID iD[1], Helena J Maier ORCID iD[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]The University of Bristol, Bristol, United Kingdom

Abstract

As enveloped viruses, coronaviruses (CoVs) assemble new particles in association with cellular membranes, bud through those membranes, and are released from the cell to produce progeny. The CoV envelope (E) and membrane (M) proteins are key drivers of this process, however, the cellular proteins and pathways involved aren't fully understood. Current understanding suggests that the pathways are conserved across all CoVs, therefore, a detailed understanding of the steps involved may inform the future development of novel broad-spectrum control strategies. We have identified chemical inhibitors of endoplasmic reticulum-Golgi transport which reduce progeny particle release, showing different levels of inhibition across three Infectious Bronchitis virus (IBV) strains representing different genotypes with a range of pathogenicity and tropism phenotypes. Ongoing work aims to pinpoint the specific stages of replication being affected, and to also validate disruption of particle assembly/release by bioimaging techniques. We have also identified ten host vesicular trafficking proteins, via mass spectrometry, that interact with the IBV E protein, two of which have previously been identified as important for IBV replication. siRNA knockdown is being utilised to examine which of these genes are important for viral replication, the mechanism of action for hits of interest are to then be explored further with immunofluorescence and transmission electron microscopy techniques.

A015

Identification and Structural Analysis of Human Cytomegalovirus Chromatin Binding Proteins

Jingyi Chen[1], Ramasubramanian Sundaramoorthy[2], Jack Sands[1], Kyle Strachan[1], Anasua Deb[1], Christina Paulus[1], Ulrich Schwarz-Linek[1], Michael Nevels[1]

[1]University of St Andrews, St Andrews, United Kingdom. [2]University of Dundee, Dundee, United Kingdom

Abstract

The double-stranded DNA genomes of human cytomegalovirus (HCMV) and other herpesviruses associate with host cell histones to form nucleosomes during latent and lytic infection. Little is known about how HCMV proteins interact with histones to control transcription, replication, repair and packaging of viral genomes and chromatin-based processes in the host genome. We have previously shown that HCMV immediate-early protein 1 (IE1, pUL123) binds to core histones H2A, H2B, H3 and H4 and targets the nucleosome surface by interacting with H2A-H2B dimers via a conserved ten amino acid motif in its chromatin tethering domain. Using a chromatin proteomics approach, we identified several additional HCMV core histone binding proteins that were not previously known to bind to chromatin. One of these novel viral histone binding proteins is pp65 (pUL83), the major component of the HCMV tegument. Like IE1, pp65 binds to H2A-H2B in the 'acidic pocket' of the nucleosome core particle, and the nucleosome binding motif was mapped to conserved residues in the linker domain of pp65. The IE1 and pp65 nucleosome complexes were subjected to *in silico* analysis using AlphaFold 3. In addition, IE1, pp65 and the pp65 chromatin binding domain were affinity purified from *E. coli*. The purified proteins will be used for *in vitro* reconstitution of nucleosome complexes and structural analysis by cryo-EM and NMR. Preliminary results indicate that the pp65 chromatin binding domain is intrinsically disordered in the absence of histones. Our results identify new histone binding HCMV proteins and suggest novel mechanisms of chromatin control during infection.

A016

How the virus got in: Unpacking sources of transmission in HIV prevention trials with deep-sequence pathogen data – BCPP/ Ya Tsie study.

Lerato Magosi ORCID iD[1], Eric Tchetgen Tchetgen[2], Vlad Novitsky[3], Molly Pretorius Holme[3], Janet Moore[4], Pam Bachanas[4], Refeletswe Lebelonyane[5], Christophe Fraser[6], Sikhulile Moyo[7], Kathleen E. Hurwitz[8], Tendani Gaolathe[7], Ravi Goyal[9], Joseph Makhema[7], Shahin Lockman[3], Max Essex[3], Victor De Gruttola[8], Marc Lipsitch[3]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]Department of Statistics, The Wharton School, University of Pennsylvania, Philadelphia, USA. [3]Harvard T.H. Chan School of Public Health AIDS Initiative, Department of Immunology and Infectious Disease, Harvard T.H. Chan School of Public Health, Harvard University, Boston, USA. [4]Division of Global HIV/AIDS and TB, Centers for Disease Control and Prevention, Atlanta, USA. [5]Ministry of Health, Republic of Botswana, Gaborone, Botswana. [6]Oxford Big Data Institute, Li Ka Shing Center for Health Information and Discovery, Nuffield Department of Medicine, Old Road Campus, University of Oxford, Oxford, United Kingdom. [7]Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana. [8]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Harvard University, Boston, USA. [9]Division of Infectious Diseases and Global Public Health, University of California San Diego, La Jolla, La Jolla San Diego, USA

Abstract

A longstanding puzzle in efforts to control the spread of HIV, the virus that causes AIDS, is why HIV treatment-as-prevention efforts in sub-Saharan Africa showed modest reductions in the occurrence of new infections despite achieving substantial gains in viral suppression. There is no successful vaccine for HIV or widely administrable cure and it is estimated that 1.3 million people were newly infected with HIV in 2023. Therefore, limiting the occurrence of new HIV infections is a major public health concern in infectious disease. Using data from a community-randomized trial of HIV prevention in sub-Saharan Africa we offer for the first time an evidence-based explanation of this longstanding puzzle. More specifically, we deep-sequenced whole genome HIV viral sequences from 5,114 trial participants in the 30-community BCPP (Botswana Combination Prevention Project) trial in Botswana to track the directional spread of HIV infections between communities. After, we combined the deep-sequenced HIV viral genome data with population census data to quantify the relative contribution of transmissions to intervention communities from individuals in the same community, other intervention communities, control communities, and (the vast majority of the country) non-study communities. We found that individuals in non-study communities accounted for most of the transmissions to trial communities, and that the impact of the BCPP intervention in reducing transmissions to trial communities could have been considerably larger if the intervention had been applied nationwide.

A019

Drug-like Small Molecule Blockers of Human Cytomegalovirus Reactivation from Latency

Athena Ganesh[1], Denis Korolev[2], Paula Rayner[1], Annabel Davies[1], Artur Bodiu[2], Erica Appiah[1], Samuel Moulding[1], Sarah Hosgood[1], Michael Nicholson[1], Colin Crump[1], Michael Nevels[2], Emma Poole[1]

[1]University of Cambridge, Cambridge, United Kingdom. [2]University of St Andrews, St Andrews, United Kingdom

Abstract

Human cytomegalovirus (HCMV) establishes lifelong latent infections in myeloid lineage cells of most people worldwide. Reactivation of HCMV from latency is a major cause of disease in unborn children and immunocompromised or immunosuppressed individuals, including kidney transplant recipients. Currently available drugs for HCMV inhibit viral enzymes involved in the late stages of lytic infection, select for resistant virus strains, and have limited bioavailability or toxic side effects. There are no approved antivirals that target the initial 'animation' step of HCMV reactivation.

Small molecules identified in a phenotypic screen were tested for inhibition of viral replication and animation using various molecular and cellular assays, including quantitative real-time monitoring of HCMV-infected primary cells. Two drug-like molecules selectively inhibited transcription of the HCMV genome by targeting the enhancer segment in the viral major immediate-early promoter, which serves as a latent-lytic switch during viral animation. The compounds are highly effective in inhibiting HCMV replication in primary fibroblasts, renal epithelial cells, and renal tissue slices. Moreover, they prevent HCMV reactivation from CD14+ monocytes and CD34+ haematopoietic stem cells, with durability and minimal toxicity. We also provide evidence for pan-herpesvirus activity of one compound. Identification of the cellular compound targets using a Drug Affinity Responsive Target Stability (DARTS) approach is underway.

To our knowledge, these are the first compounds shown to inhibit HCMV reactivation from latently infected cells by targeting viral animation. Drugs derived from these compounds may provide a 'block-and-lock' strategy to reduce the burden of disease caused by herpesvirus reactivation in kidney transplant recipients.

A020

Characterisation and comparison of two H5N1 high pathogenicity avian influenza viruses (HPAIVs) from the UK epizootic

Sofia Riccio ORCID iD[1], Elizabeth Billington[1], Caroline J. Warren[1], Dilhani De Silva[1], Benjamin Mollett[1], Jacob Peers-Dent[1], Saumya S. Thomas[1], Cecilia Di Genova[1], Simon Johnson[1], Alexander M.P. Byrne[2], Jafar Hassan[1], Kajal Ralh[1], Kelly J. Roper[1], Holly Coombes[1], Marek J. Slomka[1], Ashley C. Banyard[1], Joe James[1]

[1]Animal and Plant Health Agency, Weybridge, United Kingdom. [2]Francis Crick Institute, London, United Kingdom

Abstract

Avian influenza viruses (AIV) are a major economic burden worldwide, capable of devastating both wild bird populations and the poultry industry. Since the 2021 clade 2.3.4.4b panzootic, two high pathogenicity AIV H5N1 genotypes, AB and BB, have emerged and dominated infections across Europe. These genotypes arose via reassortment between circulating strains, which is known to generate new AIVs of unknown pathogenicity in different avian species. AB and BB have exhibited different affinities for wild bird species, AB for anseriformes (ducks and geese) and BB for shorebirds (gulls), but both have caused infections in poultry (chickens). Therefore, we must increase our understanding of circulating AIVs to predict, prevent, and control future outbreaks.

We will present characterisation of AB and BB in duck and chicken cells, including growth curves to compare replication kinetics in different species. To investigate how the viruses compete during co-infection, we have developed a bioinformatic approach and are applying this to analyse AB-BB reassortment in both species.

Additionally, we inoculated chickens with either AB or BB before introducing contact chickens to compare their transmission in poultry. Testing of oropharyngeal and cloacal swabs showed that both AB and BB were shed from directly infected birds, with BB reaching higher peak viral titres than AB. However, transmission rates varied significantly, 100% versus 0%, with only BB shed from contact chickens.

Future work includes AB-BB co-infections in ducks, an AIV reservoir host, to investigate how different AIVs compete in vivo and, if they reassort, characterisation of novel emergent clade 2.3.4.4b AIVs.

A022

Characterization and application of lytic and lysogenic bacteriophages against *Streptococcus bovis/equinus* complex(SBSEC) isolated from Korean ruminants

Seon Young Park ORCID iD[1], Ji Hyung Kim[2], Seongwon Seo[3]

[1]Seoul National University, Seoul, Korea, Republic of. [2]Gachon University, Seongnam, Korea, Republic of. [3]Chungnam National University, Daejeon, Korea, Republic of

Abstract

Streptococcus bovis/equinus complex (SBSEC) comprises a diverse group of commensal bacteria commonly found in the gastrointestinal tract of ruminants. However, certain SBSEC species have emerged as opportunistic pathogens, causing metabolic disorders such as subacute ruminal acidosis in livestock, resulting in significant economic losses in the global livestock industry. The increasing prevalence of antimicrobial resistance in SBSEC strains has prompted the exploration of alternative biocontrol strategies, such as bacteriophages (phages). In this study, two lytic phages (vB_SbRt-pBovineB21 and vB_SbRt-pBovineS21) and one temperate phage (vB_SbS-proRumen) were isolated and characterized. These phages were found to infect various SBSEC species, including the newly-isolated *S. ruminicola*. The lytic phages (Podoviridae) and temperate phage (Siphoviridae) exhibited broad host ranges, efficient adsorption, short latent periods, large burst sizes, and potent anti-biofilm activity. Genomic analysis of vB_SbS-proRumen revealed a 38,092 bp double-stranded DNA genome with 58 predicted ORFs, sharing high similarity and conserved genetic organization with other previously predicted SBSEC prophages. The efficacy of the lytic and temperate phages in controlling SBSEC contamination was evaluated in milk and rumen fluid, demonstrating significant reductions in SBSEC counts and effects on the broader microbial community. This study provides a characterization of novel lytic and temperate SBSEC bacteriophages isolated from ruminant sources, highlighting their diversity, unique properties, and potential applications in controlling SBSEC-related issues. The findings contribute to understanding phage-host interactions within the rumen microbiome and support the development of phage-based strategies for improving food safety and animal health while promoting antibiotic-free and sustainable livestock production practices.

A023

Monoclonal antibodies against porcine respiratory coronavirus

Emily Briggs[1,2], Basudev Paudyal[1], Joseph Newman[1], Brigid Veronica Carr[1], Sarah Keep[1], Erica Bickerton[1], Christine Rollier[2], Marie Di Placido[1], Elma Tchilian[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Surrey, Guildford, United Kingdom

Abstract

Coronaviruses pose a significant global threat to human and veterinary health, with three significant outbreaks of zoonotic respiratory coronaviruses in recent years. Understanding of the antibody response to respiratory coronaviruses is essential as it offers insights into infection dynamics and can provide valuable research, therapeutic, and diagnostic tools. We have established porcine respiratory coronavirus (PRCV) infection in pigs as a robust model for studying respiratory coronavirus infection in a large animal host, enabling investigation into the pathogenesis and immune control of these viruses.

Pigs were infected with porcine respiratory coronavirus (PRCV), and viral load and antibody responses assessed. Cells from various tissues including tracheobronchial lymph nodes, spleen, and bronchioalveolar lavage were screened for spike-specific B cells. Spike-specific cells from bronchoalveolar lavage isolated 20 days post-infection were sorted by FACS and their antibodies sequenced.

The sequenced antibodies were expressed and analysed for their binding to recombinant spike proteins from three strains of PRCV, including one strain which is currently circulating. Characteristics of the binding epitopes were investigated, and antibodies were investigated for use in a range of research methods, such as western blotting and immunocytochemistry. Functional characteristics, including neutralisation, were also assessed using various immunological techniques.

These antibodies will provide valuable research tools for studying PRCV infection, as well as establishing tools for developing and characterising monoclonal antibodies against other porcine coronaviruses.

A025

Development of an enhanced GFP-based hepatitis E virus sub-genomic replicon for live-cell imaging of viral replication

Khadijah Mohammed Abualsaoud

University of Leeds, LEEDS, United Kingdom

Abstract

Hepatitis E virus (HEV) is an emerging pathogen with significant global health impacts and limited treatment options. Studying the viral replication dynamics remains challenging, in part due to the requirements for high-containment facilities to handle infectious virus and lack of laboratory reagents and tools. To address this, we have developed a novel HEV sub- genomic replicon (SGR) system encoding StayGold, an enhanced derivative of GFP, enabling real-time visualisation and analysis of viral replication in live cells. The StayGold GFP signal offers improved sensitivity over other GFP reporters, allowing for more precise detection of viral replication from a continuous population. Replication can be quantified simply by live-cell imaging and analysis, providing a rapid and facile alternative to RT-qPCR or luciferase assays.

Transfection of liver cell lines with HEV SGR-derived transcript RNA enables detailed observation of virus replication dynamics using automated live-cell imaging with fluorescence quantitation. Replication of the StayGold SGR was readily detected by fluorescence, whilst the signal from replication incompetent forms of the genome was >100-fold lower. Interestingly, the SGR showed that replication kinetics were dependent on the cell density: at high density peak fluorescence was reached at 72 h, while at lower density the peak of fluorescence was delayed by 24 h. Work is underway to use this system for fluorescence-activated cell sorting and subsequent transcriptomic and proteomic profiling of cell populations that actively support HEV replication, aiming to uncover key transcriptional and gene expression signatures that are associated with efficient viral genome replication.

A026

Synthetic pyridylpiperazine and benzenesulfonamide derivatives: Unleashing the broad-spectrum antiviral activity against different viral families

Natasha Marques Cassani ORCID iD[1,2], Natalie J. Kingston ORCID iD[1], Ana Clara Cassiano Martinho ORCID iD[3], Thibault J. W. J. Dit Lapierre ORCID iD[3], Douglas Davison da Silva Oliveira ORCID iD[3], Celso de Oliveira Rezende Júnior ORCID iD[3], Nicola J. Stonehouse ORCID iD[1], Mark Harris ORCID iD[1], Ana Carolina Gomes Jardim ORCID iD[2]

[1]School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom. [2]Laboratory of Antiviral Research, Institute of Biomedical Sciences, Federal University of Uberlândia, Uberlândia, Brazil. [3]Laboratory of Synthesis of Drug Candidates, Institute of Chemistry, Federal University of Uberlandia, Uberlândia, Brazil

Abstract

Introduction: RNA viruses continue to pose a persistent threat for potential pandemics due to their high rate of evolution. Many significant viral outbreaks in the past decade have been caused by single-stranded RNA viruses, which can share common mechanisms of genome replication. As the global risk of viral outbreaks rises, broad-spectrum antiviral strategies are increasingly essential. Here, we evaluated synthetic pyridylpiperazine and benzenesulfonamide derivatives for their inhibitory effects on two distinct viruses: SARS-CoV-2 and Enterovirus A71 (EVA71) replication. **Methods:** An existing SARS-CoV-2 subgenomic replicon (SGR) expressing Nano-luciferase was used, however for EVA71 a new SGR derived from genogroup B2 strain MS/7423/87 harboring the Firefly luciferase reporter gene in place of the viral P1 protein was generated. In vitro transcribed RNA was transfected into A592-AT or HeLa cells respectively. For antiviral assays, synthetic compounds were screened for cytotoxicity, and the highest non-cytotoxic concentration was selected to evaluate their effect on SARS-CoV-2 and EVA71 replication. **Results:** Three compounds (ACCM129, ACCM133 and ACCM139) were found to inhibit the SARS-CoV-2 SGR by 69.5%, 51.8% and 49.6%, respectively, while for EVA71 a related compound ACCM144 inhibited replication by 43%. **Conclusion:** The results found here underscore the potential of these compounds as promising therapeutic agents against this range of emerging viral threats, paving the way for further broad-spectrum antiviral development. Further studies will better define the role of these compounds against other viral families.

A027

Understanding the mechanisms employed by two emerging flaviviruses to induce vascular leakage

Emma Brown ORCID iD[1,2], Justin Duruanyanwu[2], Kevin Maringer ORCID iD[1], Paola Campagnolo ORCID iD[2]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Surrey, Guildford, United Kingdom

Abstract

Alkhumra haemorrhagic fever virus (AHFV) and Kyasanur forest disease virus (KFDV) are two emerging zoonotic orthoflaviviruses responsible for causing haemorrhagic fever in infected patients. During orthoflavivirus infection, the viral non-structural protein NS1 is secreted into the bloodstream. NS1 can cause the endothelial barrier to become permeable, resulting in vascular leakage and haemorrhage. However, it is not understood whether AHFV and KFDV NS1 can cause vascular leakage in the same manner as other orthoflaviviruses.

The transendothelial electrical resistance (TEER) assay and electric cell-substrate impedance sensing (ECIS) assay were utilized to measure the permeability of human umbilical vein endothelial cells (HUVEC) or liver sinusoidal endothelial cell (LSEC) monolayers in monoculture and in co-culture with human stellate cells (liver pericytes). Impedance was measured over time in response to treatment with 1 µg/mL of AHFV or KFDV NS1. It was also investigated how treatment with AHFV and KFDV NS1 affected the formation of vascular structures and organization of microvascular cells, using the Geltrex angiogenesis assay.

The results showed that AHFV and KFDV NS1s affect the crosstalk between endothelial cells and pericytes in the context of vessel formation. However, AHFV and KFDV NS1 had no apparent effect on the permeability of HUVEC and LSEC monolayers in either a monoculture or co-culture system as measured by TEER and ECIS assays. These results suggest AHFV and KFDV use different mechanisms to induce vascular leakage than other orthoflaviviruses, which has implications for the development of therapeutic interventions and highlights the need for further study in this area.

A029

Investigating the role of sulfated sialylglycans in avian influenza virus infection

Samuel Jones[1,2], Jessica Quantrill[1,3], Jack Hassard[3], Jiayun Yang[1], Chris Ruis[4], Munir Iqbal[1], Tom Peacock[1], Stuart Haslam[3]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Bath, Bath, United Kingdom. [3]Imperial College, London, United Kingdom. [4]University of Cambridge, Cambrdige, United Kingdom

Abstract

The natural host reservoir of influenza viruses is wild waterfowl and seabirds. However, influenza can cross into domestic poultry such as chickens or mammals. Many subtypes and lineages of avian influenza virus have spent decades evolving endemically within farmed poultry across the world, with one particular widespread example being H9N2 viruses which are endemic in poultry across Asia, Africa and the Middle East.

A number of contemporary H9N2 strains share a preference for binding a sulfated version of the typical α2-3-linked siaylated glycan receptor; this preference is determined by a few key amino acid substitutions in haemagglutinin. We have previously found this preference may also be linked to a greater propensity to adapt to infect humans. We have more recently found that several other subtypes of poultry-adapted influenza viruses also show a preference for sulphated sialylglycans.

Therefore, in this project we aim to understand the biological significance and molecular basis of these receptor preference switches and how they may be related to changes in virus tissue tropism and host range. To do this we are using biophysical receptor binding assays, glycomics, as well as pseudovirus and live virus replication in primary and continuous cell systems, combined with overexpression or inhibiton of enzymes involved in sulfation of host cell sialylglycans.

A031

Investigating panzootic H5N1 avian influenza virus polymerase adaptations from diverse mammalian species.

Jessica Quantrill ORCID iD[1], Thomas Peacock[1], Wendy Barclay[1], Vidhi Dholakia[2], Daniel Goldhill[2], Maryn Brown[1], Joe James[3]

[1]Imperial College London, London, United Kingdom. [2]Royal Veterinary College, London, United Kingdom. [3]APHA, Surrey, United Kingdom

Abstract

In recent years, there has been unprecedented circulation of highly pathogenic H5N1 avian influenza virus of clade 2.3.4.4b across the world, originating from Europe in 2020, before spreading to every continent except Australasia. This clade has caused mass die-offs in wild birds and poultry, but most concerningly, has also caused countless incursions into both wild and farmed mammals. Two of the most notable and concerning sustained mammal-to-mammal transmission clusters have been in aquatic mammals in South America and Antarctica, and in dairy cattle in North America. In both of these cases, the virus has acquired mutations in it's polymerase that allow it to replicate efficiently in mammalian hosts.

Here, we identify mammalian adaptations acquired by clade 2.3.4.4b viruses which have successfully infected a range of mammalian species including cattle, marine mammals, farmed mink and domestic cats, and validate the impact of these mutations on polymerase activity using *in vitro* minigenome assays. We show that these viruses usually acquire mutations in their PB2 and PA segments, and that these mutations drive increased polymerase activity in human cells compared to the avian precursor polymerases. This work allows us to better understand adaptation of clade 2.3.4.4b viruses in different mammalian hosts, and helps to assess the risk these viruses pose to both humans and other mammalian species.

A032

Biological characterization of a mycovirus infecting the oilseed rape pathogenic fungus Leptosphaeria biglobosa

SIDRA HASSAN ORCID iD[1], Jacob Locke-Gotel[2], Robert Henry Arnold Coutts[2], IOLY KOTTA-LOIZOU[1,2]

[1]IMPERIAL COLLEGE LONDON, LONDON, United Kingdom. [2]University of Hertfordshire, HATFIELD, United Kingdom

Abstract

***Brassica napus*, commonly known as oilseed rape, is an economically important agricultural crop and the world's key source of vegetable oil. *B. napus* is susceptible to various diseases, including phoma stem canker caused by two fungal species of *Leptosphaeria, L. biglobosa* and *L. maculans. L. biglobosa* strain WW1 was found to be infected with Leptosphaeria biglobosa quadrivirus-1 (LbQV1), a member the family *Quadriviridae*. Following curing of LbQV-1 using the protein synthesis inhibitor cycloheximide and re-introduction of LbQV-1 into the virus-cured strain, different time course experiments were performed using these isogenic lines to assess the effects of LbQV-1 on its host. LbQV-1 affects the host phenotype, with virus-infected and virus-free cultures exhibiting different morphology and pigmentation. A direct comparison of growth on different solid and liquid media revealed that LbQV-1 infection increases radial growth and biomass production. Further experiments will focus on LbQV-1 effects on *L. biglobosa* virulence and *B. napus* systemic resistance against pathogenic microorganisms. In the future, deliberate inoculation of *B. napus* plants with hypervirulent *L. biglobosa* may help mitigate the severity of phoma stem canker and other diseases.**

A033

Examining the serological protection against Chikungunya virus in Old World Non-Human Primates through pathological assessment.

Joanna Hall, Deborah Ferguson, Claire Ham, Daniel Yara, Sarah Kempster, Neil Almond

MHRA, Potters Bar, United Kingdom

Abstract

Chikungunya virus (CHIKV) is a mosquito-borne RNA alphavirus that can cause joint swelling and chronic arthralgia in humans. Various studies have utilised non-human primates (NHPs) to investigate the progression of CHIKV disease and examine novel CHIKV vaccine immunogenicity.

We have previously established a cynomolgus macaque model to examine the protection conferred by (i) convalescent plasma, or (ii) serum from participants who have received one of two candidate CHIKV vaccines, prior to challenge with CHIKV ESCA strain LR2006-0PY1. To complement serological and molecular studies on blood samples we performed immunohistochemical examination on the Spleen, Mesenteric Lymph Node (MLN) and Inguinal Peripheral Lymph Node (iPLN) and quantified viral RNA in tissue by RT-PCR.

We observed the absence of detectable CHIKV in tissues when macaques were administered immune serum pools containing high anti-CHIKV IgG titres. By contrast, when macaques were administered with pools containing lower levels of anti-CHIKV IgG only partial protection was observed in the blood and associated with the absence of viral RNA in MLN but the detection of viral RNA in the spleen and iPLN.

These data demonstrate that irrespective of whether anti-CHIKV antibodies are derived from convalescent or vaccinated participants, macaques maybe protected from CHIKV infection. Furthermore, titratable levels of protection are associated with variable levels of virus detectable in tissues depending on anti-CHIKV titres on day of challenge.

A034

Establishing a correlate of protection against Chikungunya virus using different anti-CHIKV serological material

Daniel Yara ORCID iD, Lucy Afford, Joanna Hall, Claire Ham, Adrian Jenkins, Deborah Ferguson, Sarah Kempster, Neil Almond

Medicines and Healthcare products Regulatory Agency, Potters Bar, United Kingdom

Abstract

Approximately 75% of the global population are at risk of contracting Chikungunya virus (CHIKV). Whilst rarely lethal, a significant proportion of CHIKV patients develop chronic debilitating disease. The development of an anti-CHIKV vaccine is critical in conferring long-term protection against disease. However, the immunological correlate of anti-CHIKV levels needed to confer protection remains poorly defined although serological immunity alone is sufficient to protect in animal models and this has been used to support vaccine development.

Due to the sporadic nature of CHIKV outbreaks, regulators have accepted data from serological efficacy studies in animal models to license the first CHIKV vaccine. These studies established a neutralisation titre of 1:150 for protection, whereas studies of convalescents suggested a titre of 1:10 was sufficient.

Here, we compared protection in cynomolgus macaques challenged with 10e5 infectious units of CHIKV ESCA strain LR2006-OPY1 after administration of either (i) convalescent plasma or (ii) serum from volunteers administered with one of two candidate CHIKV vaccines. Clear titratable protection was observed with both convalescent plasma and vaccine immune serum pools, conferring different levels of protection against subsequent CHIKV challenge. Whilst peptide microarray analysis identified different antibody repertoires in each pool, we will present that semi-quantitative assays for binding and neutralising antibodies are able to identify common measures that correlate with protection.

Establishing a robust *in vitro* correlate of immune mediated protection in human sera irrespective of how antibodies were raised will accelerate the development and regulatory approval of further vaccines against this debilitating disease.

A035

Impact of Serum from Wuhan SARS-CoV-2 Vaccinated Patients on Immune Pressure in Virus Replication within the Lungs of Naïve Hamsters

Claire Ham, Jo Hall, Daniel Stubbert, Adrian Jenkins, Daniel Yara, Sarah Kempster, Deborah Ferguson, Neil Almond

MHRA, Potters Bar, United Kingdom

Abstract

Vaccines against SARS-CoV-2 have dramatically reduced disease and morbidity associated with COVID. However immunological pressures also drive evolution of the virus, forcing vaccines to be updated to prevent immunological escape.

Immune protection against severe disease correlates with serological responses, especially virus neutralising antibodies. We therefore wished to investigate the impact of serological response on virus evolution in the upper and lower respiratory tract in the hamster model of SARS-CoV-2 infection and disease.

We administered serum from patients vaccinated with the first AstraZeneca and Pfizer vaccines, followed by a challenge after a 24-hour interval with 10^6 TCID50 of the B.1.617.2 variant. Naïve hamsters were also infected to serve as a control group.

Daily weight measurements were recorded for all hamsters, and oral swabs were collected over the course of the infection. At termination, samples from both the lower and upper respiratory tracts were obtained for molecular analysis, and lung tissues were preserved using formalin fixation and paraffin embedding (FFPE).

Molecular analysis of oral swab samples showed similar levels of replication in treated and control groups, however histopathological scoring revealed that the lungs of naïve animals exhibited greater inflammation compared to those in the vaccinated groups.

We are sequencing the spike gene in viral RNA recovered from respiratory tract samples of naïve and partially protected hamsters to establish whether the rapidly replicating virus at day 9 post infection has evolved under immune pressure delivered by the Wuhan based vaccine

A036

Pasteurisation temperatures effectively inactivate influenza A viruses in milk

Jenna Schafers[1], Caroline Warren[2], Jiayun Yang[3], Junsen Zhang[4], Sarah Cole[4], Jayne Cooper[2], Karoline Drewek[2], Reddy Kolli[3], Natalie Mcginn[2], Mehnaz Qureshi[3], Scott Reid[2], Thomas Peacock[3], Ian Brown[3], Joe James[2], Ashley Banyard[2], Munir Iqbal[3], Paul Digard[1], Ed Hutchinson[4]

[1]Roslin Institute, The University of Edinburgh, Edinburgh, United Kingdom. [2]Department of Virology, Animal and Plant Health Agency-Weybridge, Surrey, United Kingdom. [3]The Pirbright Institute, Surrey, United Kingdom. [4]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

In late 2023, a high pathogenic avian influenza virus (HPAIV) H5N1 lineage was identified circulating in American dairy cattle, with high viral titers detected in raw milk. This raised a critical public health concern that milk could be a route of human infection. Pasteurization is routinely used in cow's milk to ensure safety for human consumption, but the effectiveness of it remained uncertain in influenza viruses. To assess this, we evaluated heat inactivation in milk for a panel of different influenza viruses. This included human and avian influenza A viruses (IAVs), an influenza D virus that naturally infects cattle, and recombinant IAVs carrying contemporary avian or bovine H5N1 glycoproteins. At pasteurization temperatures of 63°C and 72°C, we found that viral infectivity was rapidly lost and became undetectable before the times recommended for pasteurization (30 minutes and 15 seconds, respectively). We then showed that an H5N1 HPAIV in milk was effectively inactivated by a comparable treatment, even though its genetic material remained detectable. We conclude that pasteurization conditions should effectively inactivate H5N1 HPAIV in cows' milk, but that unpasteurized milk could carry infectious influenza viruses

A037

“Viral news” - Babies and Moms share more than just sleepless nights.

Revathy Krishnamurthy ORCID iD[1], Ryan Cook[1], Alise Ponsero[1], Hannah Pye[1], Andrea Telatin[1], David Baker[1], Sarah Philips[1], Lindsay Hall[1,2], Evelien Adriaenssens[1]

[1]Quadram Institute of Biosciences, Norwich, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom

Abstract

The maternal microbiome, including the virome component, plays a key role in shaping the infant gut microbiome, which determines the long-term health. We are investigating the maternal virome during pregnancy and the infant virome in the two first years of life using subset of samples from the Pregnancy and the Early Life Study (PEARL). The early gut virome is poorly understood, with contradictory results about the inheritance of the infant virome from maternal sources.

We have employed the NetoVIR virome enrichment protocol to capture both DNA and RNA viruses present in stool samples. After applying rigorous quality control of the sequence data, we observed 3,531 vOTUs across all individuals in our study, with approximately 69%, 15%, 12%, and 3% of vOTUs belonging to the realms *Duplodnaviria*, *Monodnaviria*, *Unclassified*, and *Riboviria*, respectively. Only a negligible fraction of vOTUs belong to the realm *Varidnaviria*.

The vOTUs count in infants appears to be variable during the initial weeks of development and reaches stability around six months. We observed that the virome is unique to individuals from early stages of development. Our results revealed shared vOTUs between mother and baby across viral realms in 93.3% of the mother-infant pairs we analysed. A few shared vOTUs were persistent in stool samples from the mother’s second trimester till the infant’s toddler stage. Our work is ongoing as we continue to explore the “what, why, and how” of shared and unique vOTUs and their role in the early virome establishment in humans in ecology and taxonomy perspectives.

A038

Dogs as potential mixing vessels of influenza A viruses

Hanting Chen[1,2], Callum Magill[1], Savitha Raveendran[1], Kieran Dee[1], Verena Schultz[1], Maria Afonso[2], Pablo Murcia[1]

[1]Medical Research Council-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [2]School of Biodiversity, One Health & Veterinary Medicine, University of Glasgow, Glasgow, United Kingdom

Abstract

Influenza emergence poses a constant public health threat. The 2009 pandemic was caused by a swine-origin virus carrying genomic segments of avian, swine, and human origin. We want to assess the risk of dogs acting as potential sources of new influenza A viruses(IAVs). Dogs are natural hosts to two canine influenza viruses (CIVs): equine-origin H3N8 and avian-origin H3N2. As dogs are highly exposed to humans and their viruses, there is a possibility that human and canine IAVs could reassort. Using an ex vivo canine tracheal explant system, we investigated the susceptibility of the canine respiratory tract to evolutionarily distinct human IAVs. We infected canine tracheal explants with human IAVs and determined their infection phenotype. As a comparator, we infected explants with CIVs. For each virus we determined the growth kinetics using plaque assays. Cytopathology and virus spread within the respiratory epithelium was assessed using histological and immunostaining approaches. To determine dog exposure to IAVs, we performed ELISA assays on ~800 canine serum samples collected between 2021 and 2024. Our results indicate that the canine respiratory epithelium is susceptible to H1N1 but not H3N2 human IAVs. Notably, we observed that a more recent H1N1 IAV is associated with higher ability to infect canine tracheal explants. We are currently using reverse genetics approaches to identify the genomic segments associated with this phenotype. Our ELISA screening showed that ~1% of the serum samples examined are positive for IAV antibodies. Our results suggests that dogs could potentially become mixing vessels of novel IAVs.

A039

Characterisation of functional RNA elements in astrovirus genomes

Ksenia Fominykh ORCID iD, Valeria Lulla

University of Cambridge, Cambridge, United Kingdom

Abstract

Astroviruses are largely understudied despite being highly prevalent enteric viruses, with their clinical impact, especially that of non-classical neurotropic strains like MLB and VA/HMO. Astroviruses are small, non-enveloped, positive-sense single-stranded RNA viruses whose complex internal RNA structures can manipulate cellular metabolism and orchestrate viral replication, creating regulatory mechanisms that support the astrovirus life cycle. The identification of functional RNA elements is crucial for understanding virus replication, packaging and host-pathogen interactions. Our recent studies underscore the importance of both sequence and structural conservation within viral RNA genomes, revealing that certain RNA structures are pivotal for viral fitness and pathogenicity. This understanding is essential for advancing antiviral strategies and vaccine design. Here, through SHAPE-MaP chemical probing and RNA structure predictions, we identify genome regions with evidence of structural conservation and investigate these functional RNA elements using astrovirus replicon and reverse genetics systems. Experimentally defining structural conservation across related RNA virus genomes is expected to uncover new RNA-mediated functions and provide valuable insights into contributors to viral replication and potential targets for impairing viral fitness.

A040

Investigation of novel methods to study the survival of foot-and-mouth disease virus in aerosols.

Charlotte Reston[1,2], Eva Perez[2], Jonathan Reid[1], Allen Haddrell[1], Simon Gubbins[2]

[1]University of Bristol, Bristol, United Kingdom. [2]The Pirbright Institute, Surrey, United Kingdom

Abstract

Foot-and-mouth disease virus (FMDV) causes a highly contagious disease of cloven-hooved animals which can cause devastating effects on the livestock and agriculture industry. The high rates of mortality in the young, the increase in abortions in infected animals, and trade restrictions for FMDV-positive countries make this a highly economic disease; annually, the cost of FMDV is approximately £5.4-17.3 billion. Aerosol transmission of FMDV is a low probability-high consequence event that has been linked to several outbreaks of the disease. Not much is known about pathogen decay rates within these inhalable particles, or what conditions are most favourable in this state, despite their importance for defining quarantine zones during active outbreaks.

This project aims to study FMDV survival within aerosols under differing environmental conditions using custom built instrumentation. The CELEBS instrument (Controlled Electrodynamic Levitation and Extraction of Bioaerosols onto a Substrate) allows virus-containing aerosols to be suspended under carefully controlled conditions, such as a chosen temperature or relative humidity. By following these levitations with infectivity assays, the impact of the environmental conditions on the aerosol and subsequently the virus particles can be characterised. This investigation will provide survival parameters for contemporary strains of FMDV, and allow the strains to be compared. These results can determine if quarantine zones around infected farms are appropriate and account for the risk of aerosolised transmission. This can ultimately better inform outbreak policy and result in more effective control of FMDV.

A041

Use of Ionising Radiation for Development of Inactivated Vaccines Against Zika Virus and Respiratory Syncytial Virus

Tom Moore[1,2], Raissa Prado Rocha[1], Lindsay Broadbent[1], Giuseppe Schettino[2], Christine Rollier[1]

[1]University of Surrey, Guildford, United Kingdom. [2]National Physical Laboratory, Teddington, United Kingdom

Abstract

Zika Virus (ZIKV) and Respiratory Syncytial Virus (RSV) are enveloped RNA viruses with challenges for developing vaccines against them. Both viruses increase infant mortality rate. There are three licensed RSV vaccines and none approved against ZIKV.

Inactivated vaccines relying on chemicals cause damage to protein-based antigens needed for protective immune responses. Ionising radiation has been understudied as a method of inactivation due to past expense. Radiation can damage viral RNA without destroying key protective antigens and is thus an interesting alternative inactivation method.

This project investigates the ability of various modalities of ionising radiation to inactivate viruses such as ZIKV and RSV to develop effective vaccines.

ZIKV and RSV were exposed to varying doses of radiation to determine the optimal conditions for inactivation. Effective radiation doses were ascertained by viral titration. The inactivated vaccine candidates were characterized by RT-qPCR, confocal microscopy, and ELISAs. Furthermore, RSV was irradiated in the presence of ROS scavengers to evaluate if this approach increased the amount of conserved pre-Fusion (F) protein.

Data indicate that both viruses are inactivated at a radiation dose of 16-20kGy. Preservation of protein expression and conformation was confirmed for RSV's F protein and ZIKV's Envelope protein. Confocal microscopy demonstrated that inactivated RSV can attach to and enter HEp2 cells, suggesting natural conformation of the F protein, and the virus' ability to bind to and enter host cells after irradiation.

ZIKV and RSV can be inactivated by gamma and electron radiation and these methods of inactivation show potential for vaccine formulation.

A042

Transcriptomic analysis of JC Virus Infection in Kidney and Brain Organoids

Si Jie Tang[1,2], Stephen Leon-Icaza[1], Colin Crump[1]

[1]Department of Pathology, University of Cambridge, Cambridge, United Kingdom. [2]University of California, Davis SOM, Sacramento, USA

Abstract

Progressive multifocal leukoencephalopathy (PML) is an often-fatal opportunistic infection of the central nervous system (CNS) caused by the human polyomavirus JC (JCV). PML can occur in the setting of hematological malignancies or any compromise of cellular immunity (HIV infection/AIDS, genetic immune deficiencies, or in patients undergoing immune suppressive treatment). Currently there are no validated treatments for PML. Initial infection with JCV leads to life-long, asymptomatic persistent infection in the kidney. When a host becomes immunocompromised, this creates an environment conducive to uncontrolled JCV replication which enables the viral genome to undergo complex rearrangements, leading to the formation of a neurotrophic virus that can efficiently infect cells in the CNS to cause PML. Due to restricted JCV host tropism and the difficulty of maintaining primary oligodendrocytes cultures, it has been difficult to model this disease *in vitro*. The development of iPSC-derived 3D organoids represents a technological advancement that may overcome these historical challenges and may serve as a model to probe the complex host-viral interactions following JCV infection. We plan to perform single-cell RNA-sequencing on kidney and brain organoids following infection with the archetype JCV variant (which underlies asymptomatic persistent infection in the immune competent host) and the neurotropic JCV. We hypothesize there will be differences in the transcriptomic environments of these organoids that may underlie differential cell tropism of archetype and prototype viral variants. Deeper understanding of viral-host interactions during polyomavirus infection could have great impact for the development of disease therapies for PML.

A043

Influenza A virus susceptibility to MX1 and BTN3A3

Udeet Ranjan Das[1], Mohammad Khalid Zakaria[1], Siddarth Bakshi[1], Nancy Liu[2], Monika Folwarczna[1], Joseph Hughes[1], Pablo Murcia[1], Matthew Turnbull[1], Massimo Palmarini[1]

[1]University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [2]Imperial College London, London, United Kingdom

Abstract

Understanding the molecular determinants and the underlying mechanisms of influenza A virus (IAV) cross-species transmission is of utmost importance for pandemic risk assessment. One barrier the virus must overcome is the evasion of host restriction factors. Human MxA (MX1) and BTN3A3 both target viral nucleoprotein (NP) that avian-origin IAVs must evade to efficiently adapt to humans. BTN3A3 evolved in old world monkeys, whereas most vertebrates have an MX1 gene. Whilst MxA restriction of avian IAVs has been well studied, the antiviral properties of MX1 from many species has either not been tested or only against limited number of IAV strains. In this study, we examined the antiviral effect of BTN3A3 and a variety of MX1 orthologs against zoonotic IAVs to explore host patterns of restriction. We generated a panel of 6:2 and 4:4 viruses, with internal segments from IAVs of various origins, and assessed them in cells overexpressing BTN3A3 or MxA/MX1. Sensitivity to BTN3A3 was largely correlated with the previously described NP residues 52 and 313. For Mx1, we found that bovine MX1 appeared to exhibit a broader antiviral activity than human MxA, inhibiting viruses that have acquired escape mutations to human MxA. Futhermore, whilst the region of NP targeted by BTN3A3 and MX1/MxA may overlap, some viruses displayed sensitivity to human MxA, but not BTN3A3. Work is ongoing to assess MX1 orthologues from additional host species, and NP mutants to further refine the determinants of MX1 and BTN3A3 restriction.

A044

Shaping Microbial Communities: The Role of Viruses in Anaerobic Digestion

Priscila Carlon ORCID iD[1], Rupsmita Sarma[1], Carl Samuel[1], Tom Curtis[1], Jan Dolfing ORCID iD[2], Mathew Brown ORCID iD[1]

[1]Newcastle University, Newcastle Upon Tyne, United Kingdom. [2]Northumbria University, Newcastle Upon Tyne, United Kingdom

Abstract

This study investigates the impact of virus-host interactions on microbial community structure, activity, and functionality within anaerobic digesters, highlighting a novel ecological role for bacteriophages. By manipulating viral predation pressures across different microbial communities, the study examines how viral dynamics influence microbial diversity and biogas production. Results show that reactors inoculated with a high abundance of indigenous viruses demonstrated increased biogas production and diversity, suggesting that viral predation may enhance functional redundancy and microbial resilience. In contrast, exposure to foreign, non-native viruses or reduced viral populations led to decreased methane output and microbial stability. These effects suggest that viral interactions help maintain the balance of key microbial groups essential for methane generation. The study further explores the role of viruses in facilitating microbial community adaptation through mechanisms like the "killing-the-winner" hypothesis, which theorises that viral predation regulates dominant microbial populations, thus sustaining diversity. Despite limitations due to experimental sensitivity, findings underscore the critical role of viruses in engineered microbial systems, potentially informing future wastewater treatment strategies to optimize microbial efficiency and stability. Further research, particularly in viral metagenomics, is recommended to elucidate the mechanisms driving these interactions.

Session : Genetics and Genomics Forum

A047

Genomic characteristics of Listeria monocytogenes causing intraamniotic infection via hematogenous dissemination

Pisut Ponghaikul ORCID iD, Pornpun Vivithanaporn ORCID iD, Piya Chaemsaithong ORCID iD

Mahidol University, Bangkok, Thailand

Abstract

Intraamniotic infection is a leading cause of preterm labour and early neonatal infection. In addition to the ascending migration of the pathogen from the urogenital tract, hematogenous migration is another possible route of transmission of the pathogen from the maternal blood circulation to the intraamniotic environment. *Listeria monocytogenes* is a common bacteria causing *Listeriosis* in immunocompromised hosts, including pregnancy. Intra-amniotic infection caused by *L. monocytogenes* is thought to be transmitted via maternal blood circulation. However, the molecular evidence to support the hematogenous route is scarce.

Whole genome sequencing using hybrid genome assembly was utilized to characterize the genomic features, including virulence factors and antimicrobial resistance, in two clinical isolates recovered from amniotic fluid and chorioamniotic membranes.

Results: *L. monocytogenes* was detected in amniotic fluid and chorioamniotic membranes, but not in vaginal fluid. Whole genome sequencing revealed that the microorganisms isolated from amniotic fluid and chorioamniotic membranes corresponded to *Listeria monocytogenes* sequence type 1, clonal complexes 1, and serotype 4b. Comparative genomic analysis illustrated similar DNA sequences of the two genomes. In addition, Browns and Hopps and Warthin-Starry staining demonstrated intracellular rod-shaped bacterial cells in the placental villi.

Conclusion: The presence of genetic similarity genome of cultivars obtained from amniotic fluid and chorioamniotic membranes with the presence of bacteria in the villi combined with the absence of *L. monocytogenes* in the vagina suggests a possible hematogenous route of intra-amniotic infection

A048

Novel mechanism of transcription activation via DNA distortions

Ksenia Klimova, David Grainger

University of Birmingham, Birmingham, United Kingdom

Abstract

MarA is a major transcriptional regulator of intrinsic antibiotic resistance in most Enterobacteriaceae species, including E. coli. This transcriptional regulator activates genes responsible for efflux, lipid trafficking, DNA repair and many other processes when a cell is under high antibiotics pressure. To date, MarA has been shown to activate transcription housekeeping RNA Polymerase only. We identified a MarA binding site in the promoter region of the flgB operon, encoding proteins needed for flagellar biosynthesis. We also identified a previously undefined promoter, dependent on the flagellar gene specific RNA polymerase. MarA activates this promoter via an unusual mechanism, whereby MarA binding deforms the promoter -10 element, allowing RNA polymerase to unwind the DNA during transcription initiation. We suggest that MarA activates flagellar biosynthesis to help bacteria escape high concentration of antibiotics or other toxic compounds.

A049

Reference-free clustering as an epidemiological tool for Mycobacterium tuberculosis transmission analysis

Aureliana Chilengue[1], Daniel Whiley[1], Maria Domingo-Sananes[1], Conor Meehan[1,2]

[1]Nottingham Trent University, Nottingham, United Kingdom. [2]Institute of Tropical Medicine, Antwerp, Belgium

Abstract

Whole genome sequencing (WGS) of *M. tuberculosis* is a robust tool widely used in epidemiological studies to detect recent transmission events. The current gold standard pipeline has proven highly valuable in clinical and epidemiological studies, but it demands substantial computational resources, making it challenging to perform in resource-limited settings where tuberculosis is most prevalent. To address this problem, we explored reference-free tools for clustering genomes to make transmission tracking feasible in settings with limited computational resources.

In this study, we analysed over 300 *M. tuberculosis* clinical isolates from Rwanda and Illumina-simulated reads using SKA2 (Split K-mers Analysis), a reference-free method that uses split K-mers to identify SNPs between genomes and cluster them based on genetic similarity. A single K-mer size of 31 was applied to both raw sequencing (Illumina and Nanopore) and assembled data to explore the SNP distances between the genomes and subsequently identify clusters of similar isolates. The SKA2 SNP distance matrix was then compared with the gold-standard WGS SNP distance matrix to assess correlation.

Our results revealed that SKA2 has the potential to detect *M. tuberculosis* transmission clusters and showed a strong correlation between the assembled genomes SKA-2 clustering and the gold-standard WGS, suggesting it could serve as a reliable alternative for tracking transmission among *M. tuberculosis* strains. Approaches such as genome assembly are crucial for fully unlocking SKA2's potential in *M. tuberculosis* molecular epidemiology. This advancement could significantly enhance WGS-based transmission analysis, particularly in resource-limited settings.

A050

Within-host diversity and body-site translocation of nosocomial *Pseudomonas aeruginosa* infections

Lewis Fisher ORCID iD[1], Harry Thorpe[2], Jukka Corander[2,1], Josie Bryant[1]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]University of Oslo, Oslo, Norway

Abstract

Background

Pseudomonas aeruginosa is an important pathogen, notorious for causing chronic respiratory tract and nosocomial infections. There has been a recent focus on *P. aeruginosa* gut-lung translocation, where the gut is proposed as a reservoir that harbours the pathogen prior to respiratory infection. However, a quantification of body-site translocation is lacking.

Methods

Metagenomic analysis was performed on nasal, rectal, and lower respiratory tract samples from 257 patients during April-May 2020 in San Matteo Hospital. We identified *P. aeruginosa* reads in 385 samples which were deconvoluted for analysis. We explored within-host genomic diversity to understand evolution and body-site transmission.

Results

From 83 patients with multiple *P. aeruginosa* positive samples, the majority (67) were colonised with a single clone. Forty four percent of patient clones had no discernible SNPs. We observed intra-clone SNPs within 28 patients where most non-synonymous mutations occurred within AMR genes, notably in *pmrB,* the *mexCD-OprJ* operons.

For 27 patients, we observed the same clone colonising multiple body-sites indicating possible body-site translocation. To distinguish this from independent environmental acquisition, we performed simulations to estimate rates of body-site translocation. We concluded that translocation was the most probable cause of body-site sharing. Moreover, ancestral state reconstruction indicated that most translocations occurred within clades associated with respiratory tract infection, suggesting that the respiratory tract was the origin of most body-site translocations.

Conclusion

Our work indicates that body-site translocation of drug-resistant *P. aeruginosa* is extremely common in this hospital setting and that translocation direction is frequently from the lung to the gut.

A051

Genomic epidemiology of plasmids carrying the *iuc5* locus and antimicrobial resistance genes in *Escherichia coli* and *Klebsiella pneumoniae* from One Health settings

Keira Cozens[1], Marjorie Gibbon[1], Natacha Couto[2], Tiffany Taylor[1], Matthew Avison[3], Edward Feil[1]

[1]University of Bath, Bath, United Kingdom. [2]University of Oxford, Oxford, United Kingdom.
[3]University of Bristol, Bristol, United Kingdom

Abstract

Aerobactin, a plasmid-mediated siderophore encoded by the *iuc* locus, is a major contributor to virulence in *Klebsiella pneumoniae.* Whilst certain lineages of aerobactin are well studied, the epidemiology of *iuc5* remains poorly understood. Moreover, our previous work suggests that plasmids harbouring *iuc3* are acquiring antimicrobial resistance genes, which may pose a major threat to public health. Here we generate hybrid assemblies of the critical priority pathogens *E. coli* and *K. pneumoniae* from humans, animals, and the environment, to determine how frequently plasmids emerge that possess both *iuc5* and antimicrobial resistance genes.

We utilised *E. coli* and *K. pneumoniae* genome sequence data from a large One Health study conducted across Thailand, generated by the OH-DART consortium. We focused on strains carrying the plasmid-mediated virulence locus *iuc5*. Long-read sequencing was used to generate hybrid assemblies of 68 *E. coli* isolates and 2 *K. pneumoniae* isolates harbouring the *iuc5* locus. Resfinder and Kleborate were used to identify plasmids with acquired resistance genes and the *iuc5* locus, respectively.

In total we identified 230 circular plasmids from *E. coli* strains harbouring the *iuc5* locus. Of these plasmids, 70 carried *iuc5*, and 68 of these plasmids contained 1 or more antimicrobial resistance genes. These plasmids were isolated from human community samples, hospital samples, fresh markets and across several different chicken, duck, and fish farms. We also identified two *iuc5*-harbouring plasmids in *K. pneumoniae* isolated from hospital samples.

Here, we report that plasmids harbouring the *iuc5* locus and antimicrobial resistance genes are widespread across different ecological sources.

A052

Lost in Translation; reassessing bldA control of antibiotic biosynthesis in Streptomyces

John Munnoch ORCID iD, Jack Stone, Iain Hunter, Paul Hoskisson

University of Strathclyde, Glasgow, United Kingdom

Abstract

Streptomyces are responsible for the production of two-thirds of clinically relevant antimicrobial agents and understanding control of their biosynthesis will help address the challenge of antimicrobial resistance. Mutations in the *bldA* locus, which encodes a rare leucyl-tRNA (TTA), results in a complete loss of morphological development and specialised metabolite production. Thought to be caused by an inability to produce important developmental proteins containing this rare codon, our work, utilizing an RNA aptamer/fluorescent protein construct, showed that transcription and translation of a TTA codon-containing gene in both wild-type and *bldA* backgrounds can be observed at an early stage in growth when $tRNA^{bldA}$ is unavailable. These data suggest that Wobble base-pairing (WBP) may occur at TTA codons via the TTG leucyl-tRNA (UUG codon) implying that the regulation of this mechanism is not as simple as previously assumed. Using gene knockouts and our RNA aptamer/fluorescent protein construct containing synthetically introduced TTA codons, we aim to further understand this important mechanism to understand if WBP occurs and its impact on translational efficiency. In parallel, using overexpression constructs of both the TTA and TTG tRNA and a conditionally toxic TTA containing *codA* construct, we will determine the functional limits of WBP and have already shown that it's possible to complement the $bldA^{TTA}$ genetic lesion through overexpression of a WBP match. Our results suggest that our understanding of this remarkable fine-tuning regulatory mechanism is not complete and has the potential to be exploited in the biotechnology industry.

A053

HIGH THROUGHPUT TESTING FOR ASSOCIATIONS BETWEEN PHASE VARIATION STATES AND DISEASE-ASSOCIATED PHENOTYPIC TRAITS OF MENINGOCOCCAL DISEASE AND CARRIAGE ISOLATES

Mercy W. Kimani[1], Robeena Farzand[1], Abdullahi Jama[1], Neil Oldfield[2], Jay Lucidarme[3], David Turner[2], Ray Borrow[3], Luisa Martinez-Pomares[2], Samuel Sheppard[4], Christopher Bayliss[1]

[1]University of Leicester, Leicester, United Kingdom. [2]University of Nottingham, Nottingham, United Kingdom. [3]UK Health Security Agency, Manchester, United Kingdom. [4]University of Oxford, Oxford, United Kingdom

Abstract

Neisseria meningitidis, an exclusive human pathogen, asymptomatically colonises the upper respiratory tract. However, meningococci can invade and multiply systemically causing invasive meningococcal disease (IMD). Meningococcal virulence is driven by phenotypic differences that might result from genetic variation (allelic, accessory or phase variation) between lineages, sub-clones or arises during IMD.

Our recent analysis of 163 MenW cc11 meningococcal isolates demonstrated that this pathogen is an informative model organism for investigating relationships between genotype and phenotype (DOI: 10.1128/mbio.03059-24). Utilising our high throughput assays, we have obtained extensive data for phenotypic traits mimicking carriage and disease behaviours for ~300 MenY:cc23 isolates. Comparison of the disease and carriage isolates has detected significant differences in multiple phenotypes including complement sensitivity and epithelial cell adhesion. We are now testing this data for associations between variation in phenotypic traits and genetic elements. Phase variation (PV) is a process that controls expression of several meningococcal genes involved in host adaptation. We have determined PV states for several outer membrane proteins (OMPs) for all of these isolates in order to identify how PV impacts these disease-associated phenotypic traits.

Whole genome sequences are available for both UK disease and concomitant carriage isolates of this lineage. We are using a genome wide association study to detect associations between allelic variation and differences in the phenotypic traits. We will discuss progress in establishing the relative importance of PV and other types of genetic variation to differences in phenotypic variation and the disease potential of both the MenW:cc11 and MenY:cc23 lineages.

A054

Integrative omics reveals distinct metabolic and microbial signatures in broilers under gut health interventions

Gladys Maria Pangga ORCID iD[1], Anne Richmond[2], Callie Hughes[2], Nicolae Corcionivoschi ORCID iD[3], Androniki Psifidi ORCID iD[4], Dong Xia ORCID iD[4], Umer Zeeshan Ijaz ORCID iD[5], Ozan Gundogdu ORCID iD[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom. [2]Pilgrim's Europe Ltd, Craigavon, United Kingdom. [3]Agri-Food and Biosciences Institute, Belfast, United Kingdom. [4]Royal Veterinary College, London, United Kingdom. [5]University of Glasgow, Glasgow, United Kingdom

Abstract

Maintaining a well-balanced gut microbiota is essential for ensuring optimal health and performance in poultry production. Gut health strategies often address coccidiosis, a common and significant enteric disease in poultry caused by *Eimeria* species. These interventions affect the gut microbial community and are expected to influence metabolite levels, reflecting the complex and dynamic relationship between gut microorganisms and metabolic processes.

In this study, we used untargeted metabolomics via liquid chromatography-mass spectrometry (LC-MS) to compare caecal metabolite levels in birds treated with ionophores (T1) and those vaccinated against *Eimeria* species (T2). Metabolomic data were normalised using the probabilistic quotient method and analysed with Sparse Partial Least Squares Discriminant Analysis (sPLS-DA). We integrated these findings with previously obtained metagenomic data, through the Data Integration Analysis for Biomarker Discovery using Latent Components (DIABLO) algorithm in the *mixOmics* R package. We also performed pathway analysis using the *MetaboAnalystR* package.

Our results identified prenol lipids as robust markers for T1, while indole derivatives were prominent in T2. Pathways involving the amino acids tryptophan and phenylalanine were enriched in both groups. *Bacteroides fragilis* had the greatest impact on the metabolome, with positive correlations to over 240 metabolites, whereas *UBA3818* spp. (Ruminococcaceae) had significant negative correlations with 22 metabolites. Overall, our findings suggest that distinct coccidiosis control methods drive diverse metabolic responses in broilers, impacting nutrient metabolism and potentially enhancing performance and health. These results provide novel insights into gut health interventions targeting *Eimeria* species.

A055

Elucidating differential expression of Shiga toxins across *Escherichia coli* serotypes

Samantha Tucker ORCID iD, David Mark ORCID iD, Rebecca McHugh ORCID iD, Andrew Roe ORCID iD

University of Glasgow, Glasgow, United Kingdom

Abstract

Shiga toxin-producing *Escherichia coli* (STEC) are a group of enteric pathogens that carry phage-encoded Shiga toxins (Stx). The association between Stx production by STEC and renal damage was first described in 1985, and its mechanism of action has since been described. STEC can produce two types of Stx: Stx-1 and Stx-2, both toxins function identically but have distinct expression systems. Classical antibiotics activate the RecA response and upregulate expression of both toxins, so they are contraindicated in treating STEC. Consequently, there is no treatment for STEC, and patients rely on supportive therapies. This study seeks to understand more about how these toxins are expressed which could inform on the design of drugs that block Stx expression.

We curated a collection of twenty-two complete chromosomes across *E. coli* serotypes from NCBI and subsequently identified eight variations of the Stx promoter regions. The eight variants were cloned into a dual transcriptional reporter system to enable simultaneous analysis of RecA-driven RFP expression and promoter-driven GFP expression. The dual reporter has been inserted into four STEC outbreak strains and *E. coli* K-12 MG1655 to elucidate whether the bacterial strain also influences Stx expression. Results show that the bacterial strains exhibit differential activation of the Stx promoter and differential responsiveness to mitomycin C, a classical DNA damaging agent. Overall, this work seeks to unravel if there are sites of variation within the promoter that may have a critical impact on toxin expression.

A056

Overcoming *Mycobacterium tuberculosis* H37Rv reference bias: a k-mer based approach to isolate-specific masking

Daniel Whiley ORCID iD[1,2], Maria R. Domingo-Sananes ORCID iD[1], Conor J. Meehan ORCID iD[1,3]

[1]School of Science and Technology, Nottingham Trent University, Nottingham, United Kingdom. [2]Medical Technologies Innovation Facility, Nottingham Trent University, Nottingham, United Kingdom. [3]Unit of Mycobacteriology, Institute of Tropical Medicine, Antwerp, Belgium

Abstract

The *Mycobacterium tuberculosis* complex (MTBC) is monomorphic with low genetic diversity. Genome masking is routinely adopted in MTBC isolate analysis to reduce false positive variant calls in highly variable regions (e.g. PE/PPE genes). Recent studies have shown that the reference genome H37Rv (MTBC lineage 4) is likely to be over-masked, leading to the removal of true positive SNPs from analyses. Additionally, masking is H37Rv-specific, making analyses in other lineages difficult. This has led to differences in masking approaches and a lack of masking schemes suitable for other MTBC lineages, confounding comparative analyses. This also has implications for the widely accepted isolate transmission linkage using 5- and 12-SNP thresholds, as different masking schemes will create different SNP counts.

To address these issues, we have developed an automated pipeline to apply consistent mapping to any genome using a k-mer-based approach and curated read mapping parameters. By identifying regions of genomes where self-50-mer mappability is poor, we generated isolate-specific masking regions. We calculated the true pairwise SNP distances within a set of 340 complete closed genomes from across the diversity of the MTBC. We then calculated false- and true-positive SNP calls between masked paired isolates, by mapping simulated Illumina sequencing reads and fine-tuning mapping parameters.

We show that this pipeline can be applied to any MTBC isolate to create strain-specific masking files. This approach generates a minimum SNP distance between isolates, minimising false positive SNP calling, allowing consistent comparisons between isolates, opening the road towards using non-H37Rv MTBC reference genomes.

A057

Allelic variation as a driver of multi-drug resistant *Escherichia coli*

Isabelle Potterill ORCID iD, Alan McNally ORCID iD

University of Birmingham, Birmingham, United Kingdom

Abstract

Multi-drug resistant (MDR) *Escherichia coli* is a problematic cause of invasive disease in humans and animals. Resistance to multiple classes of antibiotics is severely limiting treatment options. Clones from a small number of sequence types across different phylogroups are responsible for the majority of human MDR *E. coli* infections globally. These pandemic MDR clones have emerged on multiple occasions over different timeframes, highlighting the importance of studying the evolutionary pathways that led to their success. Understanding this restricted distribution of multi-drug resistance in *E. coli* is essential if we are to predict and prevent the emergence of new clones.

Here, we examine unique patterns of selection in MDR *E. coli*. By interrogating genes under selection in diverse *E. coli* lineages, we can evaluate their influence on the success of pandemic clones. We investigated a curated collection of *E. coli* genomes spanning the phylogenetic diversity of the species to identify core genes that were most highly conserved and, conversely, the most variable within and between pandemic lineages.

We identified hyper-conserved genes, indicative of purifying selection, and hypervariable genes, suggesting selective pressures including immune evasion. From this, we uncovered lineage-specific variability in genes involved in metabolism. Allelic variation in metabolic genes could therefore be an early warning sign for MDR allowing metabolic flexibility. Overall, we can use single nucleotide polymorphism-level pangenome analysis to accelerate our understanding of the emergence and evolutionary trajectory of MDR pathogens.

A058

From Pico grams to Profiles: Use of D-MDA and Long-read Sequencing to Reveal Metagenomes in Low Biomass Samples

Louise Ellison, Seri Kitada, Lewis Fisher, Josie Bryant

Wellcome Sanger Institute, Cambridge, United Kingdom

Abstract

Deciphering microbial communities that reside in different biological niches is fundamental to our understanding of how microbiomes influence human health and disease. A major obstacle in the field of respiratory metagenomics is that samples are frequently dominated by host DNA which overwhelms the metagenome and impairs our ability to detect low abundance species. Though strategies for host DNA depletion are in existence, these invariably require high concentrations of starting material and their use can result in significant sample loss to levels insufficient for input into standard sequencing workflows.

To overcome this, we have validated the use of droplet multi-displacement amplification (D-MDA) using the Samplix Xdrop as a means to amplify pico-gram amounts of DNA with minimal species bias, which we determined by long-read sequencing using MinION (Oxford Nanopore Technology) and metagenomic analysis using Kraken. We have successfully applied this approach to low biomass BAL samples that were treated using the Molysis Basic-5 kit (Molzym), identifying key bacterial species associated with chronic lung disease.

New approaches for sequencing metagenomes from minimal DNA inputs are essential for the progression of the field. The technique we present here has the potential to become a valuable tool to enable us to extract meaningful metagenomic data from low input samples, which has the potential to accelerate microbiome research.

A060

Investigating the molecular genetic mechanisms governing pathogenic *Yersinia* species soil survival

Hannah Ford

University of Nottingham, Nottingham, United Kingdom

Abstract

Yersinia pestis is the causative agent of human plague and has evolved from its closely related ancestor *Yersinia pseudotuberculosis*. *Y. pestis* is transmitted between rodents and humans, usually *via* fleas. Sporadic outbreaks of plague are often characterised by quiescent periods which may last potentially decades during which time *Y. pestis* is not recovered from rodent or flea hosts. As *Y. pseudotuberculosis* is a soil-borne pathogen, and given the similarities between the two species it is possible that *Y. pestis* may also survive in soil, providing an explanation to how plague remerges in foci following a dormant period. Some early studies suggest that *Y. pestis* can survive in soil, however the mechanisms that enable *Y. pseudotuberculosis* and *Y. pestis* to survive is not known.

This project aims to investigate what genetic factors drive *Y. pseudotuberculosis* and *Y. pestis* to survive in soil. *Y. pseudotuberculosis* (YPIII) or *Y. pestis* (YPCO92) were incubated in soil for 24 hours, after which RNA extractions and RNA-sequencing (RNA-seq) was performed. Extended RNA-seq studies have also been performed by extracting RNA from spiked soil after one week and one month incubation to observe if transcriptome changes occur in a time-dependent manner.

Comparative gene expression for the two organisms across the time points have revealed several key genes that may be involved in soil survival and illustrates a potential additional plague transmission route via exposure to contaminated soil. This study provides an alternative explanation for how plague persists in foci, and further context to the pattern of outbreaks.

A061

Role of the single-nucleotide polymorphisms (SNPs) in the genetic mechanisms of ciprofloxacin resistance in *Pseudomonas aeruginosa*

Laura Santamaria-Rubio ORCID iD[1], Matthew Shepherd[1], Niamh Harrington[2], Kendall Cagney[2], Taoran Fu[1], Anastasia Kottara[1], James Chalmers[3], Dylan Childs[4], Jo Fothergill[2], Steve Paterson[2], Michael Brockhurst[1]

[1]University of Manchester, Manchester, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]University of Dundee, Dundee, United Kingdom. [4]University of Sheffield, Sheffield, United Kingdom

Abstract

Pseudomonas aeruginosa represents a significant clinical challenge due to its high capacity to develop different resistance mechanisms to antibiotic treatments. Sequencing studies focused on the genetic mechanisms of resistance have identified several single-nucleotide polymorphisms (SNPs) located in genes related to antibiotics targets and detoxification mechanisms. Therefore, a comprehensive study of the impact and the fitness cost of these single modifications in a controlled environment have been highly desirable but limited until recently by the genetic engineering techniques available. Lately, a new toolkit based on the CRISP-Cas9 system has been developed for genetic modification in *Pseudomonas*. This toolkit involves the combined action of the endonuclease Cas9 paired with a single-stranded DNA recombineering protein (Ssr) which would introduce a single-stranded DNA with the desired SNP modifications in *Pseudomonas* genomic DNA. The main objective is to use this toolkit to introduce SNPs previously described for clinical ciprofloxacin resistant strains into the laboratory strain *P. aeruginosa* PAO1. In addition to single SNPs, we also aim to generate multiple SNP combinations. Once the mutations are confirmed, the modified strains will be phenotyped, and used in competitive fitness studies, in the absence and presence of ciprofloxacin and other commonly used antibiotics. Mutants will also be compared with both wild type PAO1 and clinical samples. This approach will generate mutants in a standardised genetic background, allowing more precise measurements of the significance of each SNP, or combinations, in producing ciprofloxacin resistance.

A062

Characterisation of non-O157 Shiga toxin-producing *Escherichia coli* from faeces of sheep flocks in Scotland

Janet Nale ORCID iD[1], Madeleine Henry ORCID iD[1], Giles Innocent ORCID iD[2], Judith Evans ORCID iD[1], Shannon Proctor[1], Lesley Allison ORCID iD[3], Anne Holmes ORCID iD[3], Nicola Holden ORCID iD[4], Sue Tongue ORCID iD[1]

[1]Centre for Epidemiology and Planetary Health, School of Veterinary Medicine, Scotland's Rural College, Inverness, United Kingdom. [2]Biomathematics and Statistics Scotland, Edinburgh, United Kingdom. [3]Scottish E. coli O157/STEC Reference Laboratory, Dept. Laboratory Medicine, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom. [4]Department of Rural Land Use, Scotland's Rural College, Aberdeen, United Kingdom

Abstract

Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) are emerging zoonotic pathogens which cause gastroenteritis worldwide. They account for ~40% of STEC infections in Scotland, which is higher than other parts of the UK. These strains are readily carried by livestock ruminants, and we previously reported carriage of STEC O157 from sheep faecal samples obtained from abattoir and flock samples collected in 2022/23. Here, we further examined the flock samples, to ascertain the diversity of non-O157 STEC they carry and their potential association with human cases of infection. Following the screening of the 65 flock faecal samples using qPCR targeting *stx* genes, 25 samples from three flocks were processed for non-O157 STEC isolation. Fifteen of the samples yielded 32 presumptive positive isolates from two flocks. Whole genome sequence analyses revealed that all the isolates lacked the *eae* gene and belonged to seven distinct non-O157 O:H serotypes. The most dominant serotypes were O166:H28 and O87:H16, identified in 12 and seven isolates, respectively. Five isolates belonged to O146:H21, and two additional isolates each were of the O176:H4, O174:H8, 0128:H2 and O113:H4 serotypes. Two isolates of serotype O146:21 belonged to a novel sequence type. One of the flocks showed higher diversity with five serotypes (O87:H16, O166:H28, O113:H4, O128:H2 and O174:H8) identified compared to the other flock with three serotypes (O146:21, O166:H28 and O176:H4). The isolates did not cluster closely with those from human infections in Scotland suggesting that diverse non-O157 STEC are preferentially selected in ovine hosts and have low potential for zoonotic transmission.

A063

Comparison of phase variable genes in persistent carriage and disease-causing isolates of Neisseria meningitidis Serogroup W.

Deven Webster[1], Luke Green[2], Chris Bayliss[1]

[1]Department of Genetics, Genomics and Cancer Sciences, University of Leicester, Leicester, United Kingdom. [2]School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom

Abstract

Background:

Neisseria meningitidis (*Nm*), a Gram-negative diplococcus bacterium, asymptomatically colonises human nasopharyngeal surfaces but also causes septicaemia and meningitis. One genetic mechanism employed by *Nm* within the host to adapt to selective pressures is phase variation (PV). PV is a random process whereby variations in simple sequence repeats (SSRs) lead to on-off switching of particular phase-variable genes. PV can influence adaptation of *Nm* to host niches by altering expression of outer membrane proteins (OMPs) during carriage to disease transitions. This study focussed on opacity-associated proteins that mediate adhesion to host carcinoembryonic receptors (CEACAM).

Method:

Meningococci encode four phase-variable *opa* genes. Gene sequences were obtained from hybrid genome sequences derived from next-generation and long-read sequencing technologies for multiple MenW:cc11 isolates. SSR numbers were confirmed by PCR amplification and GeneScan. Bioinformatic analyses were performed to compare expression states and alleles for disease and carriage isolates to phenotypic traits.

Results and Discussion

Persistent carriage of *Nm* selects for downregulation of *opa* expression through immune selection and allelic variation reducing the immunogenic targets for generation of Nm-specific antibodies. Isolates were shown to have multiple similar or identical alleles in different expression states. These states were correlated with adhesion and biofilm measurements. This data will be discussed relative to potential contributions to immune evasion and tight adherence to host cells for disease and carriage isolates of the hyperinvasive MenW:cc11 lineage.

A064

Understanding the on switch: how a novel two-component system activates formicamycin biosynthesis

Katie Noble ORCID iD, Rebecca Devine, Matthew Hutchings

John Innes Centre, Norwich, United Kingdom

Abstract

A crucial part of overcoming antimicrobial resistance is in the development and overproduction of novel antibiotics. Isolation and genetic analysis of *Streptomyces formicae* KY5, identified the formicamycins; novel antibiotics with potent activity against drug-resistant microorganisms and a high barrier to the development of resistance. There are three know cluster-situated regulators that work cohesively to control activation, repression and export of formicamycins. A two-component system, ForGF is the main activator of the pathway, and we aimed to further elucidate its function in order to exploit its role and increase production levels of the compounds. Using surface plasmon resonance alongside gene-reporter fusion assays and qRT-PCR, we have identified the binding site of the response regulator ForF and shown the impact of ForF binding on promoter activity and transcript levels. A combination of CRISPR/Cas9 mutagenesis and biophysical analyses of purified proteins have also been utilised to characterise the interaction of the two components with one another and their surroundings. Co-immunoprecipitation has also provided further insights into the way in which an activating signal is transferred and transformed into a cellular response. We have shown that manipulating ForGF and other regulators within the cluster leads to overexpression of the formicamycins, overcoming the problem of low production under standard laboratory conditions. By exploiting this mechanism of control and altering the biosynthetic regulatory pathway we also hypothesise that rewiring similar cluster-situated two component systems could be a target for overproduction of other novel antimicrobials for clinical development.

A065

A new, scalable and lasting nomenclature for *Vibrio cholerae* lineages and for sublineages of the 7PET pandemic lineage

Avril Coghlan ORCID iD[1], Vignesh Shetty ORCID iD[1], Mathew Beale ORCID iD[1], Lia Bote ORCID iD[1], Nisha Singh[1], Stephanie McGimpsey ORCID iD[1], Florent Lassalle ORCID iD[1], Corin Yeats ORCID iD[2], David Aanensen ORCID iD[2], Matthew Dorman ORCID iD[3], Nicholas Thomson ORCID iD[1]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]University of Oxford, Oxford, United Kingdom. [3]University of Galway, Galway, Ireland

Abstract

Cholera is characterised by acute watery diarrhoea, and since 2021 there have been large outbreaks in southern Africa, Pakistan, and Haiti. In countries which suffer a cholera outbreak, genome sequencing of the *Vibrio cholerae* strains responsible can be highly informative for making key public health decisions and understanding how cholera is spreading in the region. Analysing genomic data from potential outbreaks can address two key questions relevant to public health: (i) are isolates from the 7PET pandemic lineage, which is highly infectious, virulent and capable of causing explosive outbreaks, and (ii) if 7PET, what is the 7PET sublineage, as this can shed light on the possible origin of the outbreak and its relationship to other outbreaks. To enable rapid and robust lineage classification for newly sequenced genomes, we built a PopPUNK database for *V. cholerae* based on >6000 high-quality published and curated assemblies that we previously released as Vibriowatch (in Pathogenwatch https://pathogen.watch). In addition, we are developing an *in silico* genotyping approach for assigning newly sequenced 7PET genomes to 7PET sublineages, based on a set of signature SNPs (single nucleotide polymorphisms) that we identified in 2600 high-quality published 7PET genome assemblies. Our PopPUNK database can accurately determine if a genome is 7PET and discriminate between other known *V. cholerae* lineages (e.g. Gulf Coast or the candidate species *V. paracholerae*). Our SNP-based genotyping scheme can accurately identify known 7PET sublineages such as LAT1, BD-2, etc. These provide a new scalable nomenclature for *V. cholerae* lineages and 7PET sublineages.

A066

Geminio: a Nextflow workflow to uncover bacterial copy number variations

Sarah Cameron ORCID iD, Andrew Preston

University of Bath, Bath, United Kingdom

Abstract

Copy number variations (CNVs) in bacteria are understudied due to their instability and complex nature. They arise during homologous recombination between repetitive sequences, such as transposable elements and rRNA genes, leading to the duplication of regions between them. Although maintaining extra regions of DNA is thought to incur fitness costs, in certain situations, CNVs are thought to confer advantages. One frequently observed case is when antibiotic-susceptible bacteria are exposed to antibiotics: a subpopulation harbouring amplifications of resistance genes is selected for, and the population becomes resistant. Yet, when the antibiotic pressure is removed, the population loses the resistance phenotype within 50 generations, making it undetectable by standard antibiotic susceptibility testing. This phenomenon, known as heteroresistance, is difficult and labour intensive to study and therefore it's contribution to the public health problem of antimicrobial resistance (AMR) remains largely unknown.

However, with whole genome sequencing being more accessible than ever, it may offer a solution by using read depth to estimate CNVs and their prevalence. Here, we have developed a Nextflow workflow, *Geminio*, to screen sequencing data for CNVs, cataloguing their location, length, gene content, annotations and estimation of the breakpoints. We report the preliminary results in optimising the pipeline for use with bacterial genomes, testing varied assembly types as the reference for alignment (short-read, long-read or hybrid) and using sequencing reads from different technologies at various coverage levels. This pipeline will help provide novel information on CNVs in bacterial evolution and start to unpick their relationship with AMR.

A067

amr.watch - an integrated global genomics platform for monitoring antimicrobial resistance to inform public health policy

Julio Diaz Caballero[1], Natacha Couto[1], Sophia David[1], Khalil Abudahab[1], Nabil-Fareed Alikhan[1], Corin Yeats[1], Diana Connor[1], Heather Shane[1], Matthew Holden[2], Edward Feil[3], Iruka Okeke[4], Silvia Argimón[1], David Aanensen[1]

[1]University of Oxford, Oxford, United Kingdom. [2]University of St Andrews, Edinburgh, United Kingdom. [3]University of Bath, Bath, United Kingdom. [4]University of Ibadan, Ibadan, Nigeria

Abstract

The rise in whole genome sequencing for AMR markers offers insights into the emergence and spread of resistant pathogens. However, its public health potential is underutilized due to limited analytical tools. We introduce amr.watch, an interactive platform for visualizing and exploring genomic data to inform on AMR trends and risks.

We developed amr.watch, enabling exploration of four axes of information available from or associated with publicly available genomes: variant (genotype), risk (genetic AMR determinant), time and place. The amr.watch platform incorporates global genomics data in close to real-time through continuous monitoring of public archives. Analytics are performed by Pathogen.watch using community standard methods with additional AMR-species-specific curation, focusing on priority pathogens defined by the World Health Organisation. Delivery and visualisation of this data via amr.watch facilitates interrogation of historical and current trends relating to geo-temporal variant presence and risk across countries and regions.

Amr.watch incorporates publicly available genomes from 16 priority pathogens, offering a global view of the genomic AMR landscape. Despite biases favouring high-income countries, it integrates genomic, geographical, and temporal data, enabling users to visualize high-risk variant dynamics and track the emergence and spread of AMR determinants locally and globally.

Presenting genomic data integrated with geographical, temporal, and risk factors provides a concise overview for public health. It sets the stage for future integration with phenotypic, antimicrobial usage, and climate data, aiding in decision-making and prioritizing interventions to control drug-resistant organisms.

A068

Using an "artificial fitness gradient" to unravel the effects of population-wide fitness variation on the cost of antibiotic resistance.

Jack Duxbury Barber ORCID iD, Tom Vogwill ORCID iD

University of Aberdeen, Aberdeen, United Kingdom

Abstract

Understanding microbial evolutionary dynamics is key to anticipating and countering the emergence of antimicrobial resistance (AMR). However, the disconnect between in vivo observations of the fitness cost bacteria pay to maintain resistance in the absence of antibiotics and the cost observed during in vitro evolutionary experiments remains a longstanding challenge in AMR research. One potential explanatory factor for this discrepancy is the wide variation in fitness within "real-world" bacterial populations which could produce a corresponding variation in the cost of antibiotic resistance and the lack of variation in fitness within clonal lab populations of bacteria. We aim to investigate the effect of fitness variation within bacterial populations on the cost of antibiotic resistance; to do this, we have created an "artificial fitness gradient" using classical experimental evolution techniques. By isolating populations of *E. coli* at regular time-points over a period of adaptation to unfavourable growth conditions, multiple sub-populations with variations in fitness have been generated. Each of these sub-populations will be made antibiotic-resistant, and the fitness cost of antibiotic resistance for the different sub-populations will then be quantified. We anticipate that fitter sub-populations will pay a higher cost to evolve and maintain antibiotic resistance, and less-fit sub-populations will pay a comparatively lower cost. Any such variation across the artificial fitness gradient will confirm the hypothesis that variations in fitness within bacterial populations affect the cost of antibiotic resistance and will help to unravel why in vitro experimental evolution work fails to capture the true fitness cost of antibiotic resistance.

A069

Characterization of the Bvg Regulon in *Bordetella avium* and *Bordetella hinzii*.

Giulia Sarno[1], Sarah Cameron[1], Andrew Preston[1], Louise Temple[2]

[1]University of Bath, Bath, United Kingdom. [2]James Madison University, Harrisonburg, USA

Abstract

Bordetella avium and *Bordetella hinzii* are avian respiratory pathogens which have been found to pose a significant threat to poultry, especially in turkey farms where they cause coryza, marked by coughing, nasal discharge and reduced growth rates. These infections impact animal welfare, productivity and cause ongoing challenges for avian health management.

BvgAS is a two-component system that is conserved among the Bordetella. It is a key regulator of the infection biology of *Bordetella pertussis*, the causative agent of pertussis (whooping cough) and *Bordetella bronchiseptica*, responsible for kennel cough. In these species, Bvg modulates virulence factor expression in response to environmental cues, allowing effective immune evasion and host colonisation.

The Bvg regulon is well characterised in *B. pertussis* and *B. bronchiseptica.* Here, we define the Bvg regulon of *B. hinzii* and *B. avium* using RNAseq, demonstrating similar genome-wide regulation of gene expression as occurs in *B. pertussis* and *B. bronchiseptica.* We infer the processes regulated by Bvg in *B. hinzii* and *B. avium* identifying species-specific facets among significant conservation. Comparison of the roles of Bvg-regulation in regulation of gene expression between *Bordetella* species will provide insight into the evolution of pathogenicity and adaptation towards host-specificity of infection among these bacteria.

A070

VaccinesWatch: Monitoring of vaccine targets and interventions using global genome data

Sophia David, Khalil Abu-Dahab, Corin Yeats, Wa Ode Dwi Daningrat, Natacha Couto, Julio Diaz Caballero, Nabil Fareed-Alikhan, Pranit Shinde, David Aanensen

University of Oxford, Oxford, United Kingdom

Abstract

Vaccines have been the most effective intervention in the history of medicine in decreasing the burden of infectious diseases. They also represent an essential tool for combatting increasing antimicrobial resistance. However, only four of the 24 pathogens in the 2024 WHO Bacterial Priority Pathogens List have a licensed vaccine to date and new vaccines for pathogens such as *Klebsiella pneumoniae* and extraintestinal *Escherichia coli* are urgently required. Among pathogens with licensed vaccines, there is also a need for improved and/or updated formulations, for example due to emergence of non-vaccine serotypes of *Streptococcus pneumoniae* that are not included in current polysaccharide conjugate vaccines. The increasing volumes of genomic surveillance data offers a major opportunity to inform vaccine development and monitoring efforts, yet has been underexploited to date, in part due to barriers in accessing, analysing and interpreting the data.

Here we present VaccinesWatch, a resource for assessing the prevalence and distribution of key vaccine targets using continuously updated global public genome data. Using available typing schemes, we initially provide data on 1) the *cps* loci types from *S. pneumoniae*; 2) the K- and O-antigen types from *K. pneumoniae*. We highlight key applications of VaccinesWatch including evaluating and/or re-optimising vaccine geno/serotype composition, identifying emerging geno/serotypes, monitoring the effectiveness of vaccine introduction and assessing potential vaccine evasion, and highlighting data and surveillance gaps. We aim to extend our approach to a variety of relevant vaccine targets among key pathogens that are at various stages of the vaccine development pipeline.

A071

REDEFINING THE GLOBAL PHYLOGENY OF THE GENUS AEROMONAS

Nisha Singh ORCID iD[1], Rahma Ordofa[2], Mathew A Beale[1], Chetan Thakur[3], Matthew J Dorman[4], Adrian Cazares[1], Alyce Taylor-Brown[5], Fatema-Tuz Johura[6], Mahamud-ur Rashid[6], Shirajum Monira[6], Fatema Zohura[6], Tahmina Parveen[6], Sazzadul Islam Bhuiyan[6], Marzia Sultana[6], Balvinder Mohan[3], Daryl Domman[7], Samuel Kariuki[2], Christine M George[8], Neelam Taneja[3], Munirul Alam[6], Nicholas R Thomson[1]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. [3]Post Graduate Institute of Medical Education & Research, Chandigarh, India. [4]School of Mathematical and Statistical Sciences, University of Galway, Galway, Ireland. [5]Oncology Research Group, Children's Health Research Centre, Children's Health Queensland, South Brisbane, Australia. [6]Infectious diseases division, icddr,b, Dhaka, Bangladesh. [7]Department of Internal Medicine, University of New Mexico, Albuquerque, USA. [8]Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

Abstract

Aeromonads are Gram-negative bacteria that can cause gastroenteritis, septicaemia, and wound infections. *Aeromonas* spp. are routinely misidentified as *Vibrio cholerae* in selective culture. There is a paucity of genomic data describing the *Aeromona*s genus, especially for isolates sourced from a cholera endemic country. Here we sequenced 132 *Aeromonas* genomes isolated from a household study conducted during a cholera outbreak in Dhaka, Bangladesh, and 23 *Aeromonas* genomes isolated from freshwater environs of northern India. We contextualized these with 676 publicly available Aeromonas genomes. We identified 28 *Aeromonas* species and 378 unique sequence types (STs) among 831 genomes, revealing a previously underappreciated level of phylogenetic and genetic variation amongst these genomes, particularly in the distribution of virulence and antimicrobial resistance genes. Our work provides a genomic framework with which we can understand the global diversity of *Aeromonas*. This will be of particular importance to cholera endemic regions because species within this genus frequently cohabit the same environmental niches as *Vibrio* species and are also associated with diarrhoeal disease. We also provide a detailed appraisal of the genomic diversity of *Aeromonas* spp. in Dhaka, Bangladesh and northern India. These data will support future genomic epidemiological investigations using big datasets from diverse clinical and environmental sources, to understand the emerging role of *Aeromonas* in human infections.

A072

Fungi and plant stress response and Programmed Cell Death: A cross-examination of stress family proteins *MCA* and *LON* isoforms of *Saccharomyces cerevisiae* and *Arabidopsis thaliana*

Kirsty Jones ORCID iD[1], Adeola Atobatele[1], Samina Naseeb[2], Gavin McStay[3], Willian da Silveira[4], Richard Halfpenny[1]

[1]University of Staffordshire, Stoke on Trent, United Kingdom. [2]The University of Manchester, Manchester, United Kingdom. [3]Liverpool John Moores University, Liverpool, United Kingdom. [4]University of Salford, Manchester, United Kingdom

Abstract

Abiotic stresses brought on by the climate crisis have impacted crops, seeing a decrease in yields of more than 50%. This strong abiotic stress can lead to increased plant programmed cell death (PCD) and adaptation is a major challenge. This has led to a strong incentive in the development of GMO plants resistant to stress. Nevertheless, there are a few drawbacks to plant GMO development that needs to be considered like the more complex process of genetic alteration, plant cultivation maintenance, and long life cycles. *Saccharomyces cerevisiae* is renowned for its use as a model organism for understanding more complex systems. *Saccharomyces cerevisiae* have the advantage of having a shorter generation time, simpler growth methods and easier to perform genetic manipulation with. However, very little is known about how complementary the yeast and plant model *Arabidopsis thaliana* stress responses and PCD processes are. Could fungi be used as a useful tool to examine and further understand the molecular mechanisms that underpin PCD? *MCA* and *LON* isoforms are important genes involved to PCD and stress responses. Comparative genomics, transcriptomics, protein-protein interaction (PPI) networks and mitochondrial gene interactions related to *MCA* and *LON* isoforms of both organisms are being explored. Comparisons between protein homolog similarities showed mid-range sequence conservation without a clear conservation of function. Network biological function of PPI's showed that *Saccharomyces cerevisiae MCA1* and *Arabidopsis thaliana MCA5* have conserved response of heat and PCD, with lower conservation elsewhere. Ongoing analysis is focusing on the mitochondrial function as a possible biomarker.

A073

Weak links in strong pathogens: Identifying vulnerabilities in bloodstream-associated *Salmonella*

Aisling Brady ORCID iD[1], Benjamin X. Wang[2], Lijuan Luo[1], Denise Monack[2], Jay C. D. Hinton[1]

[1]Department of Clinical Infection, Microbiology and Immunology, Institute of Infection, Veterinary & Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [2]Microbiology and Immunology, Stanford University School of Medicine, California, USA

Abstract

Salmonella enterica is a leading cause of gastroenteritis, with serovars *S*. Typhimurium and *S*. Enteritidis being the most common. Recently, these serovars have emerged as significant causes of bloodstream infection, particularly in immunocompromised individuals across sub-Saharan Africa. This shift underscores the urgent need for comprehensive studies on pathovariants such as *S*. Typhimurium sequence type (ST)313, which displays unique adaptations enabling bloodstream invasion.

This research examines genetic properties that differentiate *Salmonella* isolates causing bloodstream infections from those causing gastroenteritis, using *S.* Typhimurium model strains 4/74 (gastroenteritis) and D23580 (bloodstream). Through comparative analyses between 4/74 and D23580, we investigate specific genetic features facilitating host adaptation and enhanced virulence. Our approach employs random barcode transposon site sequencing (RB-TnSeq) under infection-relevant *in vitro* conditions, focussing on human serum exposure to simulate the bloodstream environment. Our findings revealed lineage-specific fitness differences, with *fpr* and *cyaY* mutations causing significant fitness defects in D23580, but in 4/74, disruptions in these genes had no measurable impact on fitness. *fpr* and *cyaY* play crucial roles in iron homeostasis, essential for bacterial survival in iron-limited environments like the bloodstream. This contrast suggests that 4/74 has compensatory mechanisms to maintain iron acquisition and homeostasis, which D23580 lacks, likely reflecting distinct evolutionary pressures faced by each strain.

Our results support evidence that host-restricted *Salmonella* strains tolerate loss-of-function mutations in genes that would otherwise provide redundancy in generalist strains. Our findings enhance understanding of how host adaptation influences pathogen fitness and highlight potential therapeutic targets by exploiting genetic vulnerabilities in iNTS strains.

A074

Genomic comparison of highly related *E. coli* and *K. pneumoniae* isolated from the faeces and blood of the same neonatal children hospitalized with fever in Tanzania

Richard Goodman ORCID iD[1], Sabrina Moyo[1,2], Aakash Khanijau[1], Ilinca Memelis[1], Joel Manyahi[2,3], Upendo Kibwana[3], Bjorn Blomberg[2,4], Nina Langeland[2,4], Adam Roberts[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]University of Bergen, Bergen, Norway. [3]Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, United Republic of. [4]Haukeland University Hospital, Bergen, Norway

Abstract

Blood stream infections (BSI) are a major cause of hospitalisation and death for children under the age of five in sub-Saharan Africa with Gram-negative bacteria such as *Klebsiella pneumoniae* and *Escherichia coli* among the major causative agents. These bacteria usually colonise the human gastrointestinal (GI) tract which has been identified as a reservoir for invasive infections into extra-intestinal environments such as the urinary tract and bloodstream. In this study we used comparative genomics to compare hybrid assemblies of blood and faecal isolates taken from the same patients (all neonates under 19 days old) to determine if the BSI associated bacterial isolates originated in the GI tract. Using various methods, we show that both *E. coli* and *K. pneumoniae* likely translocated from the GI tract to the blood. We also highlight key genes and mutations that are indicative of pathogenic strains capable of BSI. The comparisons at the genomic and molecular level highlight changes that contribute to the GI-blood transition within individual isolates. This is integral to understanding the pathogenesis of BSI and offers evidence of genetic alterations that can guide targeted interventions and preventive measures.

A075

Beyond *aureus:* Unveiling the SCC*mec* Landscape in Non-*aureus* Staphylococci through Novel Bioinformatic Approaches

Ciarrai Jones[1], Alison MacFadyen ORCID iD[2]

[1]University of Suffolk, Ipswich, United Kingdom. [2]The Sainsbury Laboratory, Norwich, United Kingdom

Abstract

Staphylococcal cassette chromosome *mec* (SCC*mec*) elements, which confer methicillin resistance in staphylococci, exhibit remarkable diversity with 15 described types. Non-*aureus* staphylococci can act as a reservoir for SCC*mec*, and often carry composite SCC*mec* elements. The exact prevalence, phylogenetic diversity and geographical spread of SCC*mec* in non-aureus staphylococci is unknown. *SCCmec* integrate into the genome of staphylococci at a specific site, *attB*, within the 3' end of the gene *orfX*. The recognition of *attB* is achieved by recombinases carried within the SCC*mec* element, CcrAB or CcrC. After integration two *att* sites are generated flanking the SCC*mec* element, known as *attR* and *attL*. There is evidence in the literature that the recombinases bind to slightly different recognition sites, due to the generation of divergent *attR* and *attL* sites. To that end, we built a pipeline to identify SCC*mec* carriage, extract the SCC*mec* sequence and associated *attR*/*L* sites. This allowed us to elucidate phylogenetic relationships between novel SCC*mec* elements, identify *attR*/*L* motifs associated with Ccr combinations and elucidate the diversity of non-*aureus* SCC*mec*. The findings of this work expands our knowledge of the diverse range of these important staphylococcal antimicrobial resistance determinants, that are relevant to both human and veterinary medicine. Further, it provides a comprehensive understanding of typical and atypical SCC*mec* elements, by elucidating the diversity and epidemiology within non-*aureus* staphylococci. This work also highlights the importance of Ccr type carriage within SCC*mec* and the impact this can have on integration and spread of SCC*mec.*

A076

Genomic Characterisation of Anaplasma phagocytophilum Strains from the United Kingdom and Optimisation of Metagenomic Enrichment Protocols

Sean Brierley[1], Ian Goodhead[1], Benjamin Makepeace[2], Lesley Sakyi[2], Jing Khoo[2], Alistar Darby[2], Richard Birtles[1]

[1]University of Salford, Salford, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Objectives: To generate the first complete genome representations of UK Anaplasma phagocytophilum (Ap) strains and develop optimised enrichment methodologies for sequencing of Ap directly from tissue.

Methods: Seven Ap strains isolated from ruminants were sequenced using Illumina short-read and Oxford Nanopore long-read systems. Genomic analyses included phylogenetic reconstruction, pangenomic profiling, and average nucleotide identity. Enrichment strategies encompassing differential lysis (Molzym), CpG methylation depletion (NEB), RNA bait capture (Agilent SureSelect), and adaptive sampling (ONT) were systematically evaluated on deer spleen samples infected with Ap. An optimised approach was applied to an infected shrew carrying an ecotype III strain.

Results: Phylogenomic analysis delineated UK Ap strains within the European Ecotype I cluster, while revealing potential subdivisions. The pangenome identified core and accessory genes, with ANI values suggesting species boundaries within Ap. Enrichment protocols combining Monarch HMW DNA extraction, NEB microbiome depletion and bait capture yielded optimal pathogen representation. An Ecotype III strain from a common shrew was partially captured identifying the limits of the capture technology. Linkage analysis of groEL genes supported existing ecotype classifications, whereas whole genome phylogenetics indicated potential reclassification into four epidemiologically separated species.

Conclusions: This study generated the first complete Ap genomes from the UK, providing insights into genomic diversity and phylogenetic relationships. Optimised enrichment strategies were developed for high-resolution metagenomic sequencing, overcoming challenges posed by low bacterial loads and complex metagenomic samples. Whole genome analysis suggests the European ecotypes are representative of global Ap diversity, with ANI supporting the existence of four epidemiologically separate species within Ap.

A077

GnT motifs can increase T:A→G:C mutation rates >1000-fold in bacteria

James Horton ORCID iD[1], Joshua Cherry[2], Gretel Waugh[3], Tiffany Taylor[3]

[1]Institut Cochin, Paris, France. [2]NCBI, Bethesda, USA. [3]University of Bath, Bath, United Kingdom

Abstract

Nucleotides do not mutate at equal frequencies. Instead, specific nucleotide positions can exhibit much higher mutation rates than the genomic average due to their immediate nucleotide neighbours. These 'mutational hotspots' can impact evolution by making certain adaptive mutations more likely to occur than others, yet we lack knowledge of which short nucleotide tracts create mutational hotspots. In this work, we employ experimental evolution with *P. fluorescens* and bioinformatic analysis of various *Salmonella* species to characterise a short nucleotide motif (8bp) that drives T→G mutation rates >1000-fold higher than the genomic average in bacteria. First, we experimentally show that homopolymeric tracts (≥3) of G with a 3' T frequently mutate so that the 3' T is replaced with a G, resulting in an extension of the guanine tract, i.e. GGGGT → GGGGG. We build on this finding by demonstrating that the potency of this hotspot is dependent on the nucleotides immediately flanking the G_nT tract. We find that the dinucleotide pair immediately 5' to a G_4 tract, and the nucleotide immediately 3' to the T, determine whether T→G mutation rates are ~5-fold higher than the genomic average or >1000-fold higher than the average. These results show that such T→G mutational hotspots are products of several modular nucleotide components (1-4bp in length) which each exert a significant effect on the mutation rate of the G_nT motif. This work therefore advances our ability to accurately identify the position and quantify the mutagenicity of hotspot motifs predicated on short nucleotide tracts.

A078

The evolution of Type III Secretion Systems in *Vibrio cholerae*: pathogenic or environmental tool?

Lia Bote ORCID iD[1], Stephanie McGimpsey ORCID iD[1], Nicholas Thomson ORCID iD[1,2]

[1]Wellcome Sanger Institute, Hinxton, United Kingdom. [2]London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

The Type III Secretion System (T3SS) is a molecular mechanism used by bacteria to transport proteins, termed “effectors”, into eukaryotic host cells. It is involved in the pathogenicity of many Gram-negative bacterial species through functions such as cytoskeletal rearrangement to promote host invasion, colonisation, or cytotoxicity. In *Vibrio cholerae*, the T3SS has been shown in animal models to be sufficient for intestinal colonisation and inflammatory diarrhoea, even in the absence of cholera toxin and toxin coregulated pilus, the canonical virulence factors of the **seven**th **p**andemic **E**l **T**or (7PET) lineage. As such, the T3SS has been suggested to be required for clinical disease in non-7PET *V. cholerae* infection. Here, we show the prevalence, diversity, and potential mobility of the T3SS across a large collection of genomes in *V. cholerae*, the larger *Vibrionaceae* family, and the 1.9 million bacterial genomes in the AllTheBacteria collection. We demonstrate that within *V. cholerae*, the T3SS is found only in non-7PET lineages, where it is associated with but not essential for clinical disease, and that mobility of the T3SS is linked to a mosaic fragment of the previously described Vibrio Pathogenicity Island 1 (VPI-1). Structural genes for the *V. cholerae* T3SS are almost exclusively restricted to *Vibrionaceae*, found sporadically in about one fifth of species in the family, including those that are known fish and opportunistic human pathogens. This work demonstrates across large evolutionary space that the *V. cholerae* T3SS was acquired from within *Vibrionaceae*, with a potential role in both environmental and clinical persistence.

A080

Deciphering the rules of RNA-protein regulatory interactions: Regulating ribosomal protein synthesis with *de novo* RNA-binding proteins selected from random sequence

Emily Tonge ORCID iD[1], Michelle M. Meyer[2], Arianne M. Babina[1]

[1]University of Glasgow, Glasgow, United Kingdom. [2]Boston College, Chestnut Hill, MA, USA

Abstract

RNA-binding proteins (RBPs) are integral to gene regulatory networks, undertaking key roles in essential cellular processes such as post-transcriptional regulation and ribosome synthesis. As advancements in proteomics and transcriptomics approaches continue to expand our catalogue of known RBPs, understanding how these proteins interact with RNA targets to carry out their molecular functions is increasingly important. The bacterial ribosomal protein S15 is an excellent model for examining the determinants governing RBP specificity and the co-evolution of RBPs with their RNA targets. In addition to binding to the 16S rRNA during ribosome assembly, S15 acts as a negative regulator of its own expression via interactions with mRNA structures found in the 5' untranslated region of its operon transcript. While S15 and its 16S rRNA binding site are relatively conserved among bacteria, the regulatory mRNA structures interacting with S15 vary significantly across bacterial phyla and display distinct binding profiles. In this study, we aim to isolate novel *de novo* RBPs from plasmid libraries encoding randomly-generated small open reading frames that interact with the different bacterial S15-interacting mRNA structures and demonstrate gene regulatory activity *in vivo*. Comprehensive characterisation of the selected RBPs and comparisons of their cross-specificities with the various S15-interacting mRNA structures will shed light on the parameters driving RBP specificity and the *de novo* evolution of RNA-protein regulatory interactions. These insights will improve our ability to mechanistically and functionally characterise novel RBPs and inform the design of synthetic gene regulatory circuits.

A081

High-Throughput Chemical Genetic Screening of 3,000 Salmonella enterica Clinical Isolates

Huda Ahmad[1], Georgia Williams[1], Susan Sutherland[1], Blanca Perez Sepulveda[2], Jay Hinton[2], Danesh Moradigaravand[3], Manuel Banzhaf[4]

[1]University of Birmingham, Birmingham, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]King Abdullah University of Science and Technology, Thuwal, Saudi Arabia. [4]Newcastle University, Newcastle, United Kingdom

Abstract

A major challenge in microbiology remains linking genetic information to phenotypic traits, particularly in complex pathogens like *Salmonella enterica*. Chemical genetic screening offers a high-throughput method to profile the phenotypic responses of strains under a wide range of chemical and environmental stressors. To investigate associations between genetic and phenotypic profiles, we conducted a high-throughput chemical genetic screen on 3,000 *Salmonella* clinical isolates across 200-300 unique conditions.

This approach allows us to uncover clustering patterns and interactions among strains within the same clade in their responses to stress conditions, revealing shared and distinct mechanisms of resistance and adaptation. This research generates a comprehensive dataset that maps genomic profiles to phenotypic responses in *Salmonella enterica*, providing a valuable resource to advance our understanding of pathogen diversity.

A082

Carry that weight: characterisation of the *Salmonella* Enteritidis prophage repertoire in clinical isolates associated with bloodstream infection

Blanca Perez-Sepulveda ORCID iD[1], Aisling Brady[1], Léo Debaene[1], Sarah Gallichan[2], Eleanor McCall-McGovern[1], Yan Li[1], Nicolas Wenner[1], Jay Hinton[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Abstract

Background

Non-typhoidal *Salmonella* (NTS) are typically associated with enterocolitis and linked to the industrialisation of food production. In recent years, new lineages of invasive NTS serovars Typhimurium and Enteritidis have evolved to cause about 77,000 deaths per year worldwide due to bloodstream infection, predominantly in sub-Saharan Africa. Prophages can have a significant impact on the fitness of pathogens by encoding genes related to bacterial metabolism, toxins, and virulence factors. However, the prophage repertoire of *S.* Enteritidis pathovariants is yet to be characterised.

Methods

We used a combined approach for the identification and characterisation of prophages from *S.* Enteritidis pathovariants. We used bioinformatics tools for prediction of prophage regions, that were compared between genomes of ca. 1500 isolates from the 10KSG consortium. We employed RNA-seq based transcriptomic datasets generated from infection-relevant conditions to annotate FUN (function unknown) genes. Our experimental approach used prophage deletions for further characterisation.

Results

Comparative genomics revealed conservation and specificity of the prophage repertoire across the lineages. Prophage sequences were obtained from high quality *S.* Enteritidis genomes generated using a combination of short- and long-read sequencing, and functional transcriptomics revealed structural genes and potentially novel virulence factors. We are currently using genetic tools for prophage deletions to further characterise host range and other phenotypes, such as anti-phage mechanisms, that may confer an evolutionary advantage to the pathovariant lineages associated with bloodstream infection.

Conclusion

Our findings highlight the distinct and conserved prophage repertoire in *S*. Enteritidis pathovariants associated with bloodstream infections.

A083

Pangenome analysis illuminates the evolution of capripoxvirus accessory genes

Tiffany Tobi, Meilia Prihartini, Tim Downing, Jonas Albarnaz

The Pirbright Institute, Woking, United Kingdom

Abstract

Capripoxviruses are aetiological agents of debilitating diseases in livestock, consisting of three species: *Lumpy skin disease virus* (LSDV), *Sheeppox virus* (SPPV) and *Goatpox virus* (GTPV). Each species has host specificity: LSDV preferentially infects cattle, SPPV sheep and GTPV goat. However, the role of specific capripoxvirus genes in virus tropism and host range remains to be clearly understood. We hypothesised that the accessory genome of capripoxviruses encodes key functions in host defence evasion and hence determines virus tropism, like other poxviruses. To investigate this, we conducted pangenome analysis of the three capripoxvirus species. We observed patterns of adaptive evolution that could underpin host specificity of LSDV, GTPV and SPPV but only gene composition differences for GTPV. We also observed that orthologues from LSDV and GTPV were always more closely related to each other on average, whereas SPPV orthologues were more divergent evolutionarily. To evaluate these findings experimentally, we are performing genome wide genetic screens to test the role of key capripoxvirus genes in determining host preferences. By identifying genes and mutations determining host specificity, we hope to inform on the genetic basis of capripoxvirus infection and virulence in these species.

A084

Exploring the Relationship Between Clonal Complex, Isolation Source and Antibiotic Resistance in Campylobacter jejuni

Jaime Riccomini Closa ORCID iD[1,2], Samuel A. M. Conelly[2], John D. Rodgers[2], Arnoud H. M. van Vliet ORCID iD[1]

[1]School of Veterinary Medicine, University of Surrey, Guildford, United Kingdom. [2]Department of Bacteriology, Animal and Health Agency, Weybridge, United Kingdom

Abstract

As the most common food-borne bacterial pathogen worldwide, understanding factors affecting transmission and antibiotic resistance in *Campylobacter jejuni* is an important public health objective. Multi-locus sequence typing (MLST) of *C. jejuni* can identify general population groups, known as clonal complexes (CC). These complexes can support epidemiological studies investigating predominant sources of infection and be used for surveillance of antimicrobial resistance. These studies can help identify sources and emergence of antibiotic-resistant *Campylobacter*, informing the risk assessment and risk management options for this threat to public health.

In this study, we analysed the relationship between several key *C. jejuni* CCs, isolation sources, and antibiotic resistance genotypes. Using a curated set of 15,418 genomes, we predicted resistance phenotypes to aminoglycosides, macrolides, fluoroquinolones and tetracyclines. We sorted isolates by core genome MSLT to assess whether these characteristics clustered according to CC. To account for discrepancies between the quantities of CCs, we compared the proportion of each CC in each category. CC354 and CC464 had the highest proportions of isolates resistant to two and three antibiotic classes respectively (47% and 34%). At the source level, CC61 showed the strongest association with ruminants (41%), while CC45 had the highest proportion of isolates in the environment (0.6%) and animals other than poultry and ruminants (4%). CC21 showed sub-clusters with diverse associations but lacked a pattern at the complex level. Although highlighting the need for caution when using CCs in epidemiology, our results show some clear patterns which highlight trends in *C. jejuni* antibiotic resistance and infection sources.

A085

Genomic characterisation of carbapenemase-producing *Klebsiella pneumoniae*

Lauri Tuomisto[1], Garry Blakely[1], Surabhi Taori ORCID iD[2], Prerna Vohra ORCID iD[1]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]NHS Lothian, Edinburgh, United Kingdom

Abstract

Klebsiella pneumoniae is a common cause of healthcare-acquired infections estimated to cause bacteraemia in 7.1 per 100,000 people. *K. pneumoniae* poses a major threat to global health due to the rise of carbapenem-resistant strains. Treatment can become particularly challenging as these strains often acquire multiple carbapenemases, both chromosomally and via plasmids, leading to extended drug resistance. The aim of this study was to perform genomic characterisation on 11 strains of *K. pneumoniae* isolated from patient blood cultures to identify chromosomal and plasmid-borne resistance and virulence factors. Long-and short-read sequencing were performed. Genome sequences were assembled using Unicycler, a hybrid whole-genome assembler, as well as Flye, a long-read only assembler, followed by short-read alignment with Polypolish, and the best assemblies were selected using standard quality control tools. Resistance and virulence factors were characterised using ABRicate, staramr and Kleborate. Phenotypic resistance to antibiotics was determined using EUCAST disk-diffusion methodology. Most isolates displayed resistance to cephalosporins and carbapenems, and patterns of resistance to tetracycline were also as predicted. Plasmid-borne carbapenemases and the *K. pneumoniae* characteristic chromosomal extended-spectrum beta-lactamase, bla_{SHV}, were identified in all isolates. All isolates carried the *oqxAB* and *aac(6')-Ib-cr* genes that confer fluoroquinolone resistance, and 8 isolates harboured additional mutations in *gyrA* and *parC*, which are also associated with fluoroquinolone resistance. Co-presence of carbapenem-and fluoroquinolone-resistance genes in *K. pneumoniae* has been shown to result in increased treatment failure and mortality. Genomic characterisation of invasive *K. pneumoniae* isolates can inform antimicrobial surveillance, improve patient care and support infection control.

A088

A fly in the ointment; IncX3 plasmids harbouring *bla*$_{SHV-12}$ isolated from patients and a fly in a hospital in Italy.

Marjorie J Gibbon ORCID iD[1], Natacha Couto ORCID iD[2], Harry A Thorpe ORCID iD[3], Edward J Feil[1]

[1]The Milner Centre for Evolution, Department of Life Sciences, University of Bath, Bath, United Kingdom. [2]Centre for Genomic Pathogen Surveillance, Big Data Institute, University of Oxford, Oxford, United Kingdom. [3]Department of Biostatistics, University of Oslo, Oslo, Norway

Abstract

IncX3 plasmids are widespread in *Enterobacteriaceae* and have been seen to harbour a wide range of clinically important antibiotics, including *bla*$_{SHV-12}$ (Liakopoulos *et al.*, 2018). Plasmid-mediated horizontal gene transfer is an important mechanism of the spread of *bla*$_{SHV-12}$.

We used a combination of short- and long-read sequencing to characterise 46 IncX3 plasmids harboured by *Klebsiella spp.* isolates recovered as part of a large 'One-Health' studies in Italy (SpARK) (Thorpe *et al.*, 2022). Hybrid assemblies were characterised using Abricate (Seemann, Github), Kleborate (Lam *et al.*, 2021) and phylogenetic methods. IncX3 plasmid sequences were aligned using BRIG.

Of the 46 isolates harbouring IncX3 plasmids, the majority (n=42) were *K. pneumoniae*, and four other *Klebsiella* species. The majority (n=42) were isolated from hospital patients, with the remainder from pigs (n=3) and a fly (n=1). The plasmids ranged in size from 43-65 kb, and one of 233 kb. All but three of the plasmids had only the one replicon; two had two replicons and the very large plasmid had four.

Nineteen of the IncX3 plasmids harboured *bla*$_{SHV-12}$, twelve of them from the same hospital. Ten of these were from isolates of *K. pneumoniae*, and one each from *K. aerogenes* and *K. ornithinolytica*. All were from human patients except the *K. ornithinolytica* isolate that was isolated from a fly. Homology between the plasmids from the clinical isolates and the fly isolate is consistent with plasmid spread, and a role for flies as vectors of resistance plasmids in health care settings.

A089

Variability in RNA-Seq data quality across sequencing providers can impact bacterial gene expression studies

Modupeh Betts ORCID iD, Lijuan Luo, Xiaojun Zhu, Blanca M. Perez-Sepulveda, Jay C.D. Hinton

Clinical Infection, Microbiology & Immunology, Institute of Infection, Veterinary & Ecological Sciences, University of Liverpool, Liverpool, United Kingdom

Abstract

Background

RNA sequencing (RNA-Seq) has revolutionised bacterial gene expression studies by enabling high-resolution analysis of transcriptomic changes under diverse conditions. However, RNA-Seq data is often generated by commercial providers, each employing different library preparation and sequencing protocols. This study uses a structured approach to examine RNA-Seq data quality and consistency across multiple providers and concomitant effects on *Salmonella* gene expression profiles.

Methods

Salmonella enterica serovar Typhimurium strain D23580 was cultured under two conditions: LB-rich media (early stationary phase, ESP) and minimal SPI2-inducing media (mid-exponential phase, MEP). RNA was extracted using TRIzol, and three technical replicates for each condition were sent to six providers (two in the U.S. and four in Europe). Providers applied their standard bacterial RNA-Seq protocols, including ribodepletion and DNase treatment. Data were analysed using a custom bioinformatics pipeline and DESeq2, and results were compared to previous data generated by Vertis Biotechnologie AG.

Results

Data from three providers (50%) were non-strand specific and were excluded from further analysis. The remaining three providers delivered strand-specific data, which showed general concordance in gene expression profiles. However, key differences in expression levels of certain genes included *Salmonella* pathogenicity island (SPI) virulence genes. Furthermore, all providers identified fewer small regulatory RNAs than Vertis.

Conclusion

This study reveals variability in RNA-Seq data quality across providers, impacting data consistency in bacterial gene expression studies. By standardising RNA-Seq protocols, the reliability and reproducibility of *Salmonella* regulatory and virulence gene expression can be optimised.

A090

Dissecting the biology of *Klebsiella pneumoniae* using the approach of chemical genomics

Micheal Alao

Birmingham, Birmingham, United Kingdom

Abstract

Klebsiella pneumoniae is an important human pathogen with increasing antimicrobial resistance in the clinic. Although, genome sequencing information is available for several strains of the organism, functional annotations of the genes are lagging behind. This hinders our better understanding of the biology of the organism, which is important in discovering new drug targets and tackling antimicrobial resistance. We applied chemical genomics approach of systematically assessing the fitness of ordered transposon mutant library of *K. pneumoniae* (4346 mutants) against ~200 stresses. The responsive genome, which are genes inactivated in mutants with significant responses to a stress, were 42% of the genome of *K. pneumoniae.* More so, we identified 348 genes essential for growth in different stress conditions in *K. pneumoniae.*

Additionally, functions were predicted to hundreds of hypothetical genes with the functions further validated via computational methods. For example, KPNIH1_22370 was predicted to be a membrane protein with its mutant showing reduced fitness in triton-x and correlation coefficient of 0.64 with *yidD*. Then, possible interactions were validated between KPNIH1_22370 and *yidD* on ChimeraX.

Furthermore, single mutants of *envC* and *hflC* genes showed reduced fitness in aminoglycosides while single mutants of *nuo, cyo* and *sdh* genes showed increased fitness in aminoglycosides. This suggests that with leaky membrane from the inactivation of *envC* and *hflC*, more aminoglycosides get into the cell and inhibit protein synthesis. Additionally, inactivation of *nuo, cyo* and *sdh* genes resulting in increased fitness suggests there is a reduction in aminoglycosides uptake in dysfunctional electron transport chain.

A091

Do Probiotics Alter the Resistome and Co-location of Antimicrobial Resistance Genes in Tanzanian Neonates?

Ralfh Rolex Pulmones ORCID iD[1], Sabrina J Moyo ORCID iD[2,1], Joshua Gidabayda[3], Musuveni Justine[3], Bjorn Blomberg ORCID iD[2,4], Iren Høyland Löhr[5], Claus Klingenberg ORCID iD[6,7], Nina Langeland ORCID iD[2,4], Adam P. Roberts ORCID iD[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]University of Bergen, Bergen, Norway. [3]Haydom Lutheran Hospital, Haydom, Tanzania, United Republic of. [4]Haukeland University Hospital, Bergen, Norway. [5]University of Stavanger, Stavanger, Norway. [6]University Hospital of North Norway, Tromsø, Norway. [7]University of Tromsø-Arctic University of Norway, Tromsø, Norway

Abstract

Antimicrobial resistance (AMR) is a global threat disproportionately impacting Lower-Middle-Income countries. In Tanzania, ceftriaxone is often used empirically, despite recommendations as second-line treatment, contributing to the rise of Extended-Spectrum Beta-lactamases (ESBLs) especially the prevalent *bla*$_{CTX\text{-}M\text{-}15}$. Neonates are vulnerable to AMR with their already restricted treatment options and probiotics offer an alternative non-antibiotic treatment, supplementing current regimens. We investigated the effect of a probiotic blend, Labinic™, containing three strains, on the resistome and gene co-location patterns of ESBL-positive Tanzanian neonates.

The ProRIDE clinical trial recruited 2000 neonates, providing one month of Labinic™ or placebo from birth. Rectal swabs were collected at six-weeks and six-months old and screened for ESBL-*Enterobacteriaceae*. Among 500 six-week swabs, 101 were ESBL-positive, with 97 *Escherichia coli*, 31 *Klebsiella spp.* and 3 *Enterobacter cloacae/hormachei*. At six-months only 17 neonates remained ESBL-positive, totalling 22 *E. coli* and 3 *Klebsiella pneumoniae*. Both sets were Illumina-sequenced (2x250bp PE) while isolates from the 17 neonates were additionally Oxford-Nanopore-sequenced (r.10.4.1). Genomes were assembled using Shovill or Hybracter then queried against ARG-ANNOT and PlasmidFinder using Abricate. Sequence types were called by ARIBA. Core SNPs were identified with Snippy and phylogenies calculated with IQTree. Assemblies were annotated using Bakta and genomic contexts visualised in R.

Between trial groups, no differences were observed in the resistomes though gene co-occurrences, especially with *bla*$_{CTX\text{-}M\text{-}15}$, differed. In the Probiotic group, *bla*$_{CTX\text{-}M\text{-}15}$ showed a modest increase in ARGs co-located near *bla*$_{CTX\text{-}M\text{-}15}$ with preference to chromosomal insertion. Sequence type, species and ARG diversity was also higher.

A092

Inflammation-like environments limit the loss of quorum sensing in *Pseudomonas aeruginosa*

Taoran Fu, Rosanna Wright, Danna Gifford, Christopher Knight, Michael Brockhurst

University of Manchester, Manchester, United Kingdom

Abstract

Within-host environments are complex and multidimensional, making it challenging to link evolutionary responses of colonizing pathogens to their causal selective drivers. Loss of quorum sensing (QS) via mutation of the master regulator, *lasR*, is a common adaptation of *Pseudomonas aeruginosa* during chronic infections. Here, we use experimental evolution in host-mimicking media to show that loss of QS is constrained by environmental factors associated with host inflammation. Specifically, environments combining oxidative stress and abundant free amino acids limited loss of QS, whereas QS loss was rapid in the absence of oxidative stress regardless of amino acids. Under oxidative stress, *lasR* mutations were contingent upon first decoupling regulation of oxidative stress responses from QS via mutations in the promoter region of the primary catalase, *katA*, or in the oxidative stress regulator, *oxyR*, enabling maintenance of oxidative stress tolerance. Together our findings suggest that host inflammatory responses likely delay the loss of QS whilst colonizers undergo stepwise evolution, first adapting to survive lethal stressors before responding to other nutritional selective drivers that favour loss of QS.

A093

Forge Editing: A robust alternative to CRISPR without the licensing headache.

Craig Woods ORCID iD

Forge Genetics, Nottingham, United Kingdom

Abstract

The IP landscape for CRISPR is increasingly complex, with competing claims and high licensing fees, often deterring commercial use of CRISPR-based methods. “Forge Editing” is a novel, non-CRISPR gene-editing tool that bypasses these licensing barriers while delivering high-precision knockouts and insertions.

Forge Editing has demonstrated success in multiple bacterial species, including Clostridioides difficile and Escherichia coli, achieving 28 precise knockouts and creating 8 heterologous insertion strains to date. With smaller plasmid sizes and a unique mechanism of action, Forge Editing enables reliable mutant generation, even in genetically recalcitrant species like certain Clostridia —making it especially suited for non-model bacterial systems where robust genetic tools are scarce.

Forge Genetics, founded to commercialise Forge Editing, offers contract research and out-licensing opportunities and currently collaborates with clients across pharmaceutical, biotechnology, and consumer goods sectors. We aim to demonstrate Forge Editing’s applicability across a broader range of bacterial species and welcome academic partnerships to advance this goal.

A094

A Method for High-Throughput Chemical Genomic Screening to Map Phenotypical Changes

Susan Sutherland[1], Huda Ahmed[1], Georgia Williams[1], Mathew Milner[2], Danesh Moradigaravand[2], Manuel Banzhaf[3]

[1]University of Birmingham, Birmingham, United Kingdom. [2]King Abdullah University of Science and Technology, Makkah, Saudi Arabia. [3]Newcastle University, Newcastle Upon Tyne, United Kingdom

Abstract

Chemical genomics is an emerging field with vast potential. High-throughput chemical genomic screening can profile many bacterial strains grown under multiple chemical and environmental stress conditions concurrently. This can assist in the discovery of unannotated gene functions when using a single gene mutant library due to their phenotypic response and clustering patterns.

This is a step-by-step guide from the setup to final data analysis. This will first describe the pre-testing required such as the creation of bacterial source plates; how to ensure the agar is poured consistently and selecting the right concentration of stress conditions. It further details the use of a pinning robot to achieve high-throughput processing and the imaging of the grown final bacteria colony plates. For the ease of the end user the various software used to analyse the colony growth is also described with sections on possible challenges a user may face.

A095

Species Clustering of the Viridans Group Streptococci with Whole Genome Sequencing (WGS)

Wa Ode Dwi Daningrat ORCID iD, Julio Caballero, Corin Yeats, Sophia David, Natacha Couto, Nabil-Fareed Alikhan, David Aanensen

Centre for Genomic Pathogen Surveillance, University of Oxford, Oxford, United Kingdom

Abstract

Accurately identifying a species within the viridans group streptococci (VGS) has been challenging and contentious. Misassignment of species can directly impact patients' prognosis and indirectly impact evidence-based policy. In recent years, the number of bacterial whole genomes generated has increased exponentially, which can be leveraged to answer existing questions in species boundaries. Here, we showed species clustering of VGS using WGS data. This study utilised a combined dataset of unpublished VGS whole genomes from internal resources at the University of Oxford and publicly available VGS whole genomes from the European Nucleotide Archive (ENA). Python-based scripts were utilised to extract metadata and genomes from ENA, followed by data handling and cleaning before assembly and QC. High-quality genomes that met the inclusion & exclusion criteria were included in annotation, pangenome, and phylogenetic tree inference. A total of 2,359 genomes were included in the study (83.9% [1,979/2,359] from ENA, and 16.1% [380/2,359] unpublished genomes). VGS pangenome comprised 24,915 gene families with 24,136 (96.9%) accessory genes and 779 (3.1%) core genes (CG). Comparison between assigned species reported to ENA and genomic-based species assignment showed that 75.8% (1,788/2,359) were concordant. Although with some discordance assignments (24.2% [571/2,359]), a maximum-likelihood (ML) CG-based phylogenetic tree showed well-defined group-level boundaries within VGS based on their shared core genetic contents generated from WGS. Relatively high concordance species assignment using WGS was observed. The core-gene approach showed a clear demarcation of VGS, which can be utilised to enhance the precision of intervention and data-driven responses to infection and prevention programmes.

A096

Comparative functional genomics reveals adaptive differences shaping the evolution of Salmonella Typhimurium ST313 lineage 3 in sub-Saharan Africa

Logan Rolls ORCID iD, Modupeh Betts ORCID iD, Lijuan Luo, Blanca Perez-Sepulveda ORCID iD, Jay Hinton ORCID iD

The University of Liverpool, Liverpool, United Kingdom

Abstract

Salmonella enterica serovar Typhimurium sequence type (ST) 313 is the principal cause of invasive nontyphoidal *Salmonella* (iNTS) disease in sub-Saharan Africa. Previously, multi-drug resistant ST313 lineage 2 (L2) was the predominant cause of iNTS in Malawi. Following a shift in local antibiotic guidelines, a pan-susceptible ST313 lineage 3 (L3) clone has emerged, surpassing L2 as the primary cause of iNTS in the region. This study used comparative functional genomics to investigate differences between L3 (strain BKQZM9) and L2 (strain D23580) in four infection-relevant conditions. Analysis of RNA-seq data revealed several virulence and metabolic genes that were differentially-expressed between L3 and L2. Differentially-expressed genes included the down-regulation of the *pdu* propanediol utilisation operon during anaerobic growth in L3, but not L2. Interestingly, *Salmonella* pathogenicity island 2 (SPI2) genes were significantly up-regulated at early stationary phase in L2, but not L3. Since the expression of SPI2 was similar between the isolates in a macrophage-mimicking, SPI2-inducing condition, we hypothesise that L3 could have evolved to modulate SPI2 expression within the macrophage microenvironment. Although only 647 single nucleotide polymorphisms (SNPs) separate BKQZM9 and D23580, we identified several mutations within the coding sequences of transcriptional regulators in L3, which may drive gene expression differences between L3 and L2. Our preliminary findings suggest that lineage-specific SNP differences underpin the rewiring of gene regulatory systems with implications for the evolution of *Salmonella* virulence.

A097

Identification of anti-phage hotspots in the genomes of Pseudomonas aeruginosa temperate phages

Aleksei Agapov ORCID iD[1], Anna Olina[1], Giuseppina Mariano[2], Rama Bhatia[1], Edze Westra[1], Stineke van Houte[1]

[1]Environment and Sustainability Institute, Centre for Ecology and Conservation, University of Exeter, Penryn, United Kingdom. [2]Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgo, United Kingdom

Abstract

Bacteriophages play an important role in bacterial populational dynamics through cell lysis. In response to phage predation, bacteria evolved more than a hundred variants of anti-phage defence systems (DSs). DSs often co-localize on bacterial genomes in so-called “defence islands”. This feature allows to predict novel defences based on a guilt-by-association approach. This method has been extremely fruitful in the search of novel defence systems in bacterial genomes and revealed that defence islands are often located on mobile genetic elements, such as plasmids, integrons or temperate phages. In contrast to lytic phages, temperate ones can insert themselves in the host genome and be inherited by the cell progeny. Recent studies of coliphages demonstrated that DSs often form clusters within the temperate phage genomes.

In this work we sequenced a hundred temperate phages isolated from *Pseudomonas aeruginosa* strains. Sequence analysis showed that some of them carry defence systems. We clustered these phage genomes together with publicly available *P. aeruginosa* temperate phage sequences based on their predicted proteomes and looked for genomic hotspots encoding defence genes within these clusters. Our analysis reveals such hotspots and suggests candidates for novel defence systems in *P. aeruginosa* temperate phages. This work contributes to our understanding of phage genomics and evolutionary arms race between mobile genetic elements and their hosts.

A098

Does bacterial cooperation facilitate the evolution of antibiotic resistance?

Anna Dewar ORCID iD, Laurence Belcher, Stuart West, Rosie Randall

University of Oxford, Oxford, United Kingdom

Abstract

Cooperation plays a key role in the growth and success of many bacteria. Bacteria produce and secrete a range of molecules that provide benefits to the local population of cells, and therefore act as cooperative 'public goods'. For example, laboratory experiments have shown that beta-lactamase molecules, which provide resistance to beta-lactam antibiotics, act as cooperative public goods. Cooperation evolves when the benefits are directed towards closely related cells that share the gene for cooperation. This process is called kin selection, and theory predicts it should leave signatures (footprints) in the sequences of genes for cooperation. To test this across 10 species of diverse bacteria, we compared genes involved in the production of beta-lactamases to genes for private behaviours. We tested the prediction that genes controlling for cooperative behaviours should have higher polymorphism and divergence relative to genes for private behaviours. Using this molecular population genetics approach will allow us to examine whether bacterial cooperation facilitates the evolution of antibiotic resistance.

A099

Investigating replication fork blocks, replication-transcription conflicts and replication restart dynamics in living *Escherichia coli* cells

Stelinda Peros[1], Christian Rudolph[1], Mark Leake[2], Aisha Syeda[2]

[1]Brunel university of London, Uxbridge, United Kingdom. [2]University of York, York, United Kingdom

Abstract

DNA is a fundamental molecule and its replication must be completed with high accuracy. However the replisome constantly faces many obstacles. The replication and transcription conflicts are common because both processes share the same template and lead to genomic instability. Bacteria have evolved specialised replication restart proteins, which re-recruit replisomes at points where synthesis is arrested to continue DNA replication and avoid cell death.

Here we report two *in vivo* techniques to investigate the role of restart proteins in *Escherichia coli*. Firstly, we used an array of *lacO* operator sequences, bound by the LacI repressor, as a model for blocking DNA replication. Our results suggest that the PriA-PriB restart pathway is particularly important for replication restart. Unexpectedly, we also found that the helicase activity of PriA, which differs from its restart activity, is highly important.

To study replication-transcription conflicts directly we utilised strains with an additional replication origin. Thus, the replisome initiated from this ectopic origin will experience a head-on collision with the highly transcribed *rrnH* operon. Our fluorescence microscopy results suggest that these conflicts trigger an increase of active replisomes, possibly because they trigger recombination events resulting in genomic instability.

The results from our work highlight the importance of restart proteins to resolve replication-transcription conflicts. In their absence, pathways like homologous recombination are used which threaten genomic integrity. As replication-transcription conflicts take place both in bacteria and humans, our work provides novel insights into bacterial genomic instability and is also beneficial for understanding related human diseases like cancer.

A100

Establishing sequencing metrics for genome resolution of *Pseudomonas aeruginosa* from paediatric cystic fibrosis patients using PacBio Revio high-fidelity sequencing.

Mohammad Tariq[1], Mariklairi Kiourkou[1], Malcolm Brodlie[2,3], Audrey Perry[1,3], John Perry[3], Anthony De Soyza[3,2], Andrew Nelson[1], Darren Smith[1]

[1]Northumbria University, Newcastle upon Tyne, United Kingdom. [2]Newcastle University, Newcastle upon Tyne, United Kingdom. [3]Freeman hospital, Newcastle upon Tyne, United Kingdom

Abstract

Pseudomonas aeruginosa (Pa) is an opportunistic respiratory pathogen of Cystic Fibrosis (CF) and correlates to poor health outcomes if chronically colonised. The Pa genome is awash with transposable genetic elements including temperate bacteriophages or when integrated into the bacterial chromosome prophages. Prophages transduce genes that aid positive selection for the bacteria in the lung, allowing evolution via dynamic interaction. Prophage prediction tools are used to identify bacteriophages and thus complete and high-quality assemblies are an important part of obtaining accurate predictions. Multiple contigs after assembly may mask phages at sequence breaks, or possible underlying genome rearrangements inherent in short read assemblies and low quality long read sequencing data. Complete bacterial genomes without chimera are also needed to lower false discovery rates of prophage prediction.

We here sequenced 328 Pa samples on a PacBio Revio using proprietary novel pre-release indexing, with the aim to maximise a sequence run. This study aimed to highlight the minimum required Hi-fidelity data, coverage and phred quality needed to achieve robust bacterial assembly of a single contiguous Pa genome. We resolved 279 Pa genomes into a single contiguous sequence, and we see 6Mbp assembly from as little as 10x coverage at >Q40. 32 had chimera assemblies, 7 failed assembly due to low data and 10 had <95% completeness or >5% contamination based on CheckM analysis. Multiplexing also reduces the cost per bacterial genome sequence making this technology more accessible and offering less multi-contig repositories of bacterial genome data.

A101

The crucial role of the RBS in fluorescent S. epidermidis plasmids

Emily Horsburgh[1], David Mark[1], Elisa Caberlotto[2], Massimo Vassalli[1], Andrew Roe[1]

[1]University of Glasgow, Glasgow, United Kingdom. [2]L'Oreal Paris, Paris, France

Abstract

Staphylococcus epidermidis is a commensal organism of the skin microbiome. It has evolved diverse mechanisms in order to survive in these environments through specific adhesins, sensing pathways as well as influencing colonisation of other species. However, it is increasingly being recognised as an important nosocomial pathogen. *S. epidermidis* causes invasive infections in selected groups: immunocompromised individuals, preterm neonates and patients with indwelling medical devices. Unlike *S. aureus, S. epidermidis* lacks canonical virulence factors, with its ability to form biofilms central to cause disease. In addition, *S. epidermidis* has an open pan-genome with 20% of its genome consisting of variable genes which allows it to rapidly acquire new traits to adapt outside the skin environment, namely antibiotic resistance genes.

Here, we characterise the genome of *S. epidermidis* NCIMB 8558 through Next Generation sequencing. In-depth analysis revealed that this strain does not encode Type II Restriction Modification (RM) systems. Therefore, it is a suitable candidate for genetic manipulation to study gene expression using fluorescent report systems for its role in both commensal and disease phenotypes. However, these reporter systems are hindered in *S. epidermidis* as low levels of expression are seen due to their low GC content. Furthermore, the role of the Ribosome Binding Site (RBS) in *S. epidermidis* fluorescent constructs has been shown to be a crucial factor in expression levels. Here, we have facilitated the construction of a small library of fluorescent reporter plasmids containing mCherry with different RBS to allow characterisation of the expression levels for each RBS.

A102

Microalgal Encapsulation in Droplets Using a Flow-Focusing Microfluidic Device for Growth Monitoring and Transcriptomic Analysis

Shagufta Rehmat ORCID iD[1,2], Anashanth Jeyasountharan[2], Francesco Del Giudice ORCID iD[2], Eva Sonnenschein ORCID iD[2]

[1]University of Naples Federico II, Naples, Italy. [2]Swansea University, Swansea, United Kingdom

Abstract

Microfludics offers unique opportunities for studying single-cell dynamics in controlled environments, which is particularly valuable for microalgal research owing to their importance in biofuel production, wastewater treatment, and circular economy. The microalgae microdroplets provide an isolated microenvironment that allows for precise observation of growth patterns over time, revealing cellular behavior that might be obscured in bulk culture due to population heterogeneity.

In this study, we are exploring the encapsulation of the marine microalgae *Tetraselmis suecica* in droplets using a flow-focusing microfluidic channel, to capture single cells within uniform droplets and to monitor their physiology over time. Given recent advances in controlled encapsulation with viscoelastic (non-Newtonian) fluids, we are conducting these experiments in both Newtonian and non-Newtonian fluids, which may provide greater control over droplet stability and encapsulation efficiency.

Following sufficient cell proliferation within the droplets, we will perform transcriptomic analysis to compare the gene expression profiles of the encapsulated cells with those grown in bulk cultures. This comparative analysis will offer insights into how the microenvironment within droplets, including non-Newtonian fluids influences the microalgal growth at the molecular level, revealing any adaptive responses or metabolic shifts that occur in encapsulated versus bulk conditions.

Our work aims to advance the understanding of microalgal behaviour in confined microenvironments and provide a foundation for future applications of microfluidic encapsulation in environmental biotechnology, biofuel production, and single-cell studies.

A103

Genomic insights of two seaweed-associated Bacillota strains from the Brazilian Fluminense coast

Bruno Francesco Rodrigues de Oliveira[1], Eduardo Costalonga Alves[1], Erica de Souza Monteiro[1], Marcelo Torres Alvarez[1], Marinella Silva Laport[2]

[1]Fluminense Federal Univeristy, Niterói, Brazil. [2]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Abstract

Even though seaweed holobionts are promising sources of microbial products from a biotechnological standpoint, genome mining of macroalgae microbiomes is still in its infancy. Here, we surveyed the genomes of two bioactive Bacillota strains isolated from five different macroalgae genera sampled in three coastal cities in the Rio de Janeiro state (Brazil). Two bacilli strains, *Codium*-derived B2C3CT.2 and *Ulva*-associated 17A2.5, were confirmed as producers of antimicrobial substances and carbohydrate-active enzymes, being selected for genome sequencing in the Illumina platform and genomic analyses with myriad of bioinformatic tools. Genome-based taxonomy confirmed B2C3CT.2 as *Bacillus altitudinis* and revealed 17A2.5 as a potential new species in the *Sutcliffiella* genus. *B. altitudinis* B2C3CT.2 had a genome size of approximately 3.7 Mb, GC content of 41.24%, with 3,769 estimated CDS. The genome of *Suticliffiela* sp. 17A2.5 was 4.18 Mb-long, with a GC content of 40.6%, harbouring 4,200 CDS. Genome mining revealed 10 biosynthetic gene clusters (BGCs) from six classes in the B2C3CT.2 strain, notably bacilysin, lichenysin and schizokinen, while the strain 17A2.5 had only four BGCs from three classes, particularly petrobactin and terpenoid compounds. The CAZome of both strains varied between 2.81% and 3.78% and were particularly enriched in glycoside hydrolase and polysaccharide lyase families involved with the catabolism of chitin, laminarin, starch, cellulose and pectin. Our results will guide further characterisation of these biomolecules from both seaweed-associated Bacillota strains in the hope of finding usage in different industrial contexts.

A104

Do Probiotics influence the plasmids of Extended-Spectrum β-Lactamase-producing Enterobacteriaceae?

Ralfh Rolex Pulmones ORCID iD[1], Sabrina J Moyo ORCID iD[2,1], Joshua Gidabayda[3], Musuveni Justine[3], Bjorn Blomberg ORCID iD[2,4], Iren Høyland Löhr[5], Claus Klingenberg ORCID iD[6,7], Nina Langeland ORCID iD[2,4], Adam P Roberts ORCID iD[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]University of Bergen, Bergen, Norway. [3]Haydom Lutheran Hospital, Haydom, Tanzania, United Republic of. [4]Haukeland University Hospital, Bergen, Norway. [5]University of Stavanger, Stavanger, Norway. [6]University Hospital of North Norway, Tromsø, Norway. [7]University of Tromsø-Arctic University of Norway, Tromsø, Norway

Abstract

Extended-Spectrum β-Lactamases (ESBLs) are enzymes which confer resistance to 3rd Generation Cephalosporins. The most prevalent ESBL gene in Tanzania is *bla*$_{CTX\text{-}M\text{-}15}$ where it endangers neonates, further restricting their limited antibiotic options. Probiotics offer an alternative to circumvent antimicrobial resistance, supplementing current regimens. We investigated the effect of a probiotic blend, Labinic™, containing three strains, on *Enterobacteriaceae* with ESBL-carrying plasmids from colonised neonates.

The ProRIDE clinical trial recruited 2000 neonates, providing them either one month of Labinic™ or a placebo. Rectal swabs were collected at six-weeks and six-months old and screened for ESBL-*Enterobacteriaceae*. From 500 neonates, only 17 were ESBL-positive at both timepoints, with 36 *Escherichia coli*, 8 *Klebsiella pneumoniae*, 1 *Klebsiella oxytoca* and 1 *Enterobacter cloacae.* These bacteria were Illumina (2x250bp PE) and Oxford Nanopore (r.10.4.1) sequenced. Genomes were assembled using Hybracter and annotated with Bakta. Comparative plasmid analysis was performed and visualised in Proksee.

Among the isolates, 89% carried *bla*$_{CTX\text{-}M\text{-}15}$, with 52% of these genes located on plasmids. Plasmid comparison revealed that *bla*$_{CTX\text{-}M\text{-}15}$ was more common in the Probiotic group (n = 15/24), with their plasmids carrying more antimicrobial resistance genes and had unique resistance patterns to the Control group. Plasmid diversity, even within Incompatibility groups, suggested multiple acquisition events of *bla*$_{CTX\text{-}M\text{-}15}$ and plasmid hybridisation.

A106

Unraveling the cellular processes controlled by a novel regulator in *Serratia*

Daniel Plaza, George Salmond

University of Cambridge, Cambridge, United Kingdom

Abstract

The enterobacterium *Serratia* sp. ATCC 39006 (herein *Serratia*) is considered a model for studying the biosynthesis and regulation of diverse bioactive secondary metabolites, particularly two different antibiotics—a carbapenem and prodigiosin. The carbapenem is a β-lactam, whereas prodigiosin is a red pigment with diverse promising applications. These compounds are tightly regulated in response to various physiological and environmental signals.

We aimed to identify and characterise novel regulators of secondary metabolite production by random transposon mutagenesis, using wild type (WT) pigment production (prodigiosin) for mutant screening. Transposon effects on phenotype were verified by transducing out the mutations into a WT genetic background via a generalised transducing phage. An intergenic region insertion between two convergently transcribed genes resulted in a hyperpigmented phenotype. One of these surrounding genes, coding for a transcriptional regulator, provoked contrary effects to the insertion mutant, suggesting an interplay. The characterisation of the transposon mutant and the double mutant with the regulator suggested that the latter caused the phenotype observed.

Bypass mutants of the intergenic mutation confirmed the regulator's role in antibiotic production. Interestingly, this regulator also had various effects on other traits, such as motility and virulence. Thus, we sought to characterise the global effects via proteomic analysis of the single and double mutants, enabling us to pinpoint the cellular processes affected.

Hence, we found a new regulator of the antibiotic synthesis that further controls a wide range of key adaptive mechanisms, expanding the known repertoire of global regulators that may prove useful in future applications.

A107

Exploring Soil Microbial Diversity for Novel Antibiotic Production

Jenileima Devi, Weijie Zhou, Dr Nick Allenby

University of Sunderland, Sunderland, United Kingdom

Abstract

This study investigates soil microbial diversity across desert, forest, mountain, and hot spring soils, focusing on Actinobacteria, known for producing bioactive compounds. We isolated a diverse range of bacterial strains through selective media and DNA sequencing, identifying novel species of *Nocardia* and *Streptomyces* with potential antimicrobial properties. Long-read Oxford Nanopore sequencing was used to generate high-quality genome assemblies (e.g., high N50 and low contig counts) linked to complete genomes, allowing for detailed characterisation of biosynthetic gene clusters (BGCs).

Bioactivity assays revealed that over 70% of isolates exhibited antimicrobial activity, particularly against *Bacillus subtilis* and yeast, positioning soil microbiomes as a promising source for new antibiotics. Additionally, our iChip experiment, enhanced with Rifampicin, improved Actinomycetes isolation, demonstrating the iChip's effectiveness in culturing hard-to-grow microbes.

This dataset provides a valuable foundation for advancing microbial biodiversity studies, antibiotic discovery, and sequencing technologies. The findings highlight the importance of exploring diverse environmental niches to uncover novel bioactive compounds, with potential applications in addressing the growing threat of antimicrobial resistance.

A108

Characterisation of LES prophage 6 from the Liverpool Epidemic strain of Pseudomonas aeruginosa.

Lamyaa Al qarni[1,2], Heather E. Allison[1], Jo Fothergill[1], Chloe Jams[3]

[1]Department Clinical Infection, Microbiology and Immunology, Institute of Infection, Veterinary and Ecological Sciences, Biosciences Building, University of Liverpool., Liverpool, United Kingdom. [2]Department of Biology, Faculty of Science, Bisha University,, Bisha, Saudi Arabia. [3]School of Science Engineering and Environment, The University of Salford, Salford, United Kingdom

Abstract

The Liverpool Epidemic Strain (LES) of *Pseudomonas aeruginosa,* is known to cause chronic infections in the lungs of people with cystic fibrosis that are highly transmissible and difficult to treat. Notably, the genome of clinical isolate LESB58 harbours five prophages (LESϕ2-6) that have been suggested to contribute to LES competitiveness. Most of the LES phages are unique and have been extensively characterised. However, less attention has been paid to LESϕ6, which shares considerable similarities with the filamentous phage pf4 (a pf1-like phage) already integrated into the PAO1 chromosome and has been strongly associated with biofilm formation. The purpose of this study was to employ a molecular biology approach to investigate the role that LES ϕ6 plays in promoting biofilm formation and antibiotic resistance in *P. aeruginosa.*

This study's first stage was to identify the left and right boundaries of LESϕ6 experimentally. Outward-facing primers were designed to confirm the prophage ends by amplifying the rejoined ends of the circular replication intermediate form. This amplification product was then sequenced. The next steps are to recreate a clean integration site (*attB*) in PAO1 where Pf4 currently resides. This will enable the construction of an isogenic version of PAO1 that carries LES prophage 6 in place of Pf4 to enable empirical comparisons. This work will ensure that the functions of LESϕ6 can be reliably determined in comparison to the PAO1 Pf4 deletion strain in further studies to unveil how filamentous phages influence key traits of their bacterial hosts.

Session : AMR – Mechanisms & Regulation

A109

Cathelicidin-mimicking peptoids exhibit antimicrobial efficacy against the oral pathobiont *Treponema denticola*

David Scott, Jinlian Tan, Gwyneth Lamont, Gill Diamond

University of Louisville School of Dentistry, Louisville, USA

Abstract

The use of marijuana, which contains multiple potent antimicrobials, has emerged as a risk factor for early-onset periodontitis, a dysbiosis-associated destructive disease of the oral cavity. Preliminary microbiome analyses suggest that multiple oral pathogens, including *Treponema denticola*, are promoted in the oral cavity of marijuana users. We have developed a library of membrane permeabilizing anti-microbial peptoids (sequence-specific N-substituted glycine oligomers), based on well-characterized endogenous mammalian antimicrobial peptides. We hypothesized that members of this library, due to broad-spectrum activity, may represent adjunctive agents for the control of cannabinoid-resistant oral pathobionts. The growth of *T. denticola* was monitored in the presence or absence of physiologically relevant doses of the major antimicrobial phytocannabinoids (cannabidiol [*CBD*], cannabinol, or tetrahydrocannabinol, 0-100 mg/ml) with or without of the addition of anti-microbial peptoids (A-G, 0-32 mg/ml)**.** Select antimicrobial peptoids (A, B, D, E, F) exhibited impressive anti-spirochetal activities (I.C.50 values 14 to 22 mg/ml). Unexpectedly, CBD enhanced the growth-suppressing activities of the antimicrobial peptoids. Adjunct therapies that specifically target phytocannabinoid-resistant bacteria may prove to be efficacious in the prevention or control of periodontitis in those who consume marijuana. Here we establish that several inexpensive, enzymatically stable antimicrobial peptoids efficiently inhibit *T. denticola* and may be worthy of further investigation for activity against other cannabis-promoted oral microbes. There is evidence that marijuana use also predisposes to syphilis, a contemporarily resurgent infection caused by *Treponema pallidum*. It will be of interest to determine if *T. pallidum* is similarly sensitive to components of our antimicrobial peptoid arsenal.

A110

DISTRIBUTION OF RESISTANCE GENES AMONG MULTI-DRUG-RESISTANT BACTERIAL PATHOGENS OF LOWER RESPIRATORY TRACT IN KEBBI STATE, NIGERIA

Zaharaddin Muhammad Kalgo[1], Binta Muhammad Aminu[2], Bashir Muhammed[2]

[1]Department of Microbiology, Faculty of Science,Federal University Birnin Kebbi, Birnin Kebbi, Nigeria. [2]Department of Microbiology, Faculty of Life Sciences, Bayero University Kano Bayer, Kano, Nigeria

Abstract

This study was designed to determine the distribution of resistance genes among multi-drug-resistant bacterial pathogens of Lower Respiratory Tract in Kebbi State, Nigeria. Three hundred and fifty (350) sputum samples were collected from patients attending six different hospitals in Kebbi State. The samples were all screened for bacterial pathogens using standard microbiological techniques. Antimicrobial susceptibility test was determined using disc diffusion method to detect resistant isolates including MDR isolates. The MDR bacterial isolates were subjected to molecular analysis using conventional PCR techniques to detect ESBL and carbapenemase genes using standard protocol. Most of the isolates were susceptible to piperacillin (51%), trimethoprim-sulfamethoxazole (61%), Azithromycin (70%), Ciprofloxacin (71%) and Gentamycin (74%). High level of resistance to almost all the βeta-lactam antibiotics, Macrolide (Erythromycin) and Glycopeptide (Vancomycin) were recorded. The overall incidence of multidrug-resistant (MDR) isolates in this study was 39.8%. High level of MDR were observed among Burkholderia pseudomallei 3(100%), Pseudomonas aeruginosa 5(83%) and Aeromonas hydrophila 5(83%). The most frequently detected ESBL genes among the MDR isolates were bla-TEM and bla-CTX-M 1 group genes. Most of the isolates had combination of bla-TEM + bla-CTX-M 1 group or bla-SHV + bla-CTX-M 1 group in their genomes. In conclusion, it was found out that, most of the isolates were resistance to β-lactam antibiotic tested. Azithromycin, Ciprofloxacin, Gentamycin and piperacillin remain the useful antibiotics in the treatment of LRTIs in this location. bla-TEM and bla-CTX-M 1 group genes were the most frequently detected resistance genes among the MDR isolates analyzed.

A111

Prevalence and associated risk factors of Vancomycin resistant *Enterococcus faecium* in well water used for domestic purposes in Ile-Ife, Southwestern Nigeria

Babatunde Odetoyin ORCID iD, Timothy Bebe, Ezekiel Akinkumi

Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract

Vancomycin-resistant *Enterococcus faecium* (VREf) has become a major public health concern worldwide. Although hospital-based transmission is associated with outbreaks, contaminated water may play a role in their spread. We assessed 350 domestic wells for VREf, and questionnaires were administered. Isolates were identified using biochemical and molecular methods. Their susceptibility profiles were determined by standard methods. The resistance (*vanA*, *vanB*, *msrA*/B, *mefA*, *mph* (*ABC*)) and virulence (*esp*, *gelE*, and *hla*) genes of VREf were detected by Polymerase chain reaction. Data analysis was done with R statistical software. Thirty-eight (10.9%) wells were contaminated by VREf. Wells sited near the dumpsite, with ponding within three metres and split water collection, significantly harbored VREf ($p < 0.05$). All isolates exhibited resistance to tetracycline, linezolid, penicillin, erythromycin, vancomycin, and their MIC varied from 64 to 512 ug/ml. Twenty-seven isolates haboured only *the vanA* gene, while one haboured both *the vanA* and *vanB* genes. Five isolates, three isolates, one isolate and four isolates harbored the *msrA*/B gene, the *mph(ABC)* gene, both the *mph(ABC)* and *mefA* genes, and *the mefA* gene respectively. Virulence determinants, *esp*, *gelE and hla* were found in 2.6%, 29% and 28.9% of the isolates, respectively. The presence of VREf in well water highlights the risk to human health associated with the use of untreated water. There is a need for periodic sanitation and inspection of wells to prevent ponding, split water collection, and possible outbreaks of waterborne diseases.

A112

The acquisition of antibiotic resistance-associated mutations in different genetic backgrounds

Sidra Kashif ORCID iD, Alasdair Hubbard, Maria Rosa Domingo-Sananes

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Mutations in DNA gyrase and efflux pump-mediated extrusion of the drug are predominant mechanisms that confer resistance to fluoroquinolones in Gram-negative bacteria. However, little is known about how the genetic background impacts single-step mutations that bacteria acquire to develop resistance to fluoroquinolones. Here, we isolated spontaneous single-step mutants with decreased susceptibility to ciprofloxacin from multiple clinical strains of *Escherichia coli* and *Klebsiella pneumoniae* and identified the targets of mutations with whole-genome sequencing. In three out of four strains of *K. pneumoniae,* ciprofloxacin-selected mutations were present in the transcriptional regulator of OqxAB efflux pump, while the fourth strain acquired mutation in the transcriptional regulator of AcrAB efflux pump. The inhibition of these efflux pumps with 1-(1-Naphthylmethyl) piperazine in the mutants decreased their minimum inhibitory concentration of ciprofloxacin, further confirming the pump-mediated extrusion of the drug. In contrast, all the ciprofloxacin-selected *E. coli* mutants developed mutations in DNA gyrase subunit A. No fitness loss was found to be associated with the mutations present in either efflux pump regulators or DNA gyrase in both nutrient-rich and minimal media. In addition, mutants isolated from *K. pneumoniae* showed clinical-level resistance to trimethoprim, nitrofurantoin and cefalexin; however, this collateral resistance was not present in any of the *E. coli* mutants. These results suggest that the genetic background influences the acquisition of resistance-associated mutations at both strain and species level. This study also highlights that the design of effective treatment strategies may depend on the genetic background in which antibiotic resistance-associated mutations arise.

A113

Lineage-specific response to antibiotics in *Escherichia coli*

Rebecca Hall ORCID iD[1], Freya Allen[1], Elizabeth Cummins[2], Samuel Sheppard[2], Alan McNally[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]University of Oxford, Oxford, United Kingdom

Abstract

Antibiotic resistant *Escherichia coli* is a global health concern. *E. coli* is an extremely diverse species, with antibiotic and multidrug resistance (MDR) generally restricted to certain lineages. Large events, such as MDR plasmid acquisition, are known to be extremely important for resistance. We know less however about the extent to which smaller genetic changes, including in genes not typically associated with resistance, may influence the success of a resistant lineage. We also do not understand fully why antibiotic resistance is concentrated within certain phylogroups and sequence types (STs) but not others. Here, we start to unpick this using a diverse collection of 16 *E. coli* strains from eight different STs, including ST131, ST73, and ST10. We experimentally evolved these strains in the presence and absence of three antibiotics followed by genotypic and phenotypic analyses of the evolved populations. We found little evidence of repeatable genetic changes between STs, suggesting the response to antibiotics was lineage-specific. Further, we identified significant genetic changes that resulted in increased antibiotic resistance. These occurred in genes not previously linked to resistance, and may therefore be candidate potentiating mutations that could be influential in the evolutionary trajectory of antibiotic resistant lineages.

A114

Glucose alters the evolutionary response to gentamicin in uropathogenic *Escherichia coli*

Shalini Choudhary, Jacob Smith, Alan McNally, Rebecca Hall ORCID iD

University of Birmingham, Birmingham, United Kingdom

Abstract

Urinary tract infection (UTI) is a major global health and economic concern. Uropathogenic *Escherichia coli* (UPEC) are the leading cause of UTI, and antibiotic resistant UPEC are becoming increasingly common. The microenvironment of the urinary tract is metabolically distinct, and there is growing interest in understanding the extent to which metabolism may influence UPEC infection and response to antibiotics. Diabetes, characterised in part by glycosuria, is a known risk factor for UTI and is associated with more severe infections. The role that glucose plays in driving UPEC evolution is unclear. Here, we found that a pathologically-relevant glucose concentration reduced the efficacy of the antibiotic gentamicin against a UPEC strain. Through experimental evolution, we identified mutations associated with gentamicin that were not present when populations were evolved in both glucose and gentamicin. Together, this suggests that whilst glucose might reduce antibiotic killing of UPEC, it also reduces the evolution of resistance mutations, possibly through increased efflux. This provides new avenues for understanding the evolution and treatment of UPEC-mediated UTI in high-risk individuals.

A115

Genomic Characterizations of Klebsiella variicola: Emerging Pathogens Identified from Sepsis Patients in Ethiopian Referral Hospitals

Melese Hailu Legese[1,2], Daniel Asrat[1], Adane Mihret[2], Badrul Hasan[3], Abraham Aseffa[2], Göte Swedberg[3]

[1]Addis Ababa University, Addis Ababa, Ethiopia. [2]Armauer Hansen Research Institute, Addis Ababa, Ethiopia. [3]Uppsala University, Uppsala, Sweden

Abstract

Healthcare in low- and middle-income countries is becoming problematic due to the emergence of multidrug-resistant bacteria causing serious morbidity and mortality. *Klebsiella variicola* carrying multiple antimicrobial resistance (AMR) genes mainly Extended-spectrum-beta-lactamase (ESBL) is emerging significantly among sepsis patients. Between October 2019 and September 2020, this study was conducted at four Ethiopian hospitals located in the central (Tikur Anbessa and Yekatit 12), southern (Hawassa), and northern (Dessie) parts. Among 1416 sepsis patients, blood cultures were performed from which 74 *K. variicola* isolates were identified using MALDI-TOF and subjected to whole genome sequencing. Most *K. variicola* were identified at Dessie (n=44) and Hawassa (n=28) hospitals. Most *K. variicola* strains identified at Dessie Hospital displayed phylogenetic clonality, carried an IncM1 plasmid and the majority were ST3924 while others were ST906 and novel STs. Many *K. variicola* identified at Hawassa Hospital were clonally clustered, belonged to a novel ST and carried IncFIB(K) and IncFII(K) concurrently. *aac(3)-lla, bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{LEN2,}}$ *bla*$_{\text{OXA-1}}$, *bla*$_{\text{SCO-1}}$, *catB3*, *dfrA14*, *QnrB1*, *aac(6')-lb-cr* and *sul2* were AMR genes frequently encoded by *K. variicola*. Fifty *K. variicola* carried ESBL genes while 2 isolates harbored AmpC. Virulence genes detected were *mrk operons* for biofilm formation and *iron ABC transporter operons* for siderophore uptake. *K. variicola* identified at Dessie and Hawassa Hospitals had unique capsular *wzi allele 269* and *wzi allele 582* respectively. The isolation of multidrug-resistant *K. variicola* as an emerging sepsis pathogen calls for strong infection prevention strategies and antimicrobial stewardship supported by advanced bacterial identification techniques.

A116

Ecosystem's Oxygen Deficiency and Selection of Antimicrobial Resistance Genes in a One Health Perspective

Laura Ortiz Miravalles ORCID iD[1,2], Amalia Prieto Nieto ORCID iD[1,2], Nicolas Kieffer ORCID iD[1,2], Ester Vergara Gonzalez[1,2], Alberto Hipólito Carrillo de Albornoz ORCID iD[1,2], Jose Antonio Escudero García-Calderón ORCID iD[1,2]

[1]Universidad Complutense de Madrid, Madrid, Spain. [2]VISAVET Health Surveillance Centre,, Madrid, Spain

Abstract

Bacteria must face and adapt to a variety of physicochemical conditions in the environment and during infection. A key condition is the concentration of dissolved oxygen, proportional to the partial pressure of oxygen (PO2), which is extremely variable among environmental biogeographical areas and also compartments of the human and animal body. We sought to understand if the phenotype of resistance determinants commonly found in Enterobacterales can be influenced by oxygen pressure. To do so, we have compared the MIC in aerobic and anaerobic conditions of isogenic *Escherichia coli* strains containing 136 different resistance genes against 9 antibiotic families. Our results show a complex landscape of changes in the performance of resistance genes in anaerobiosis. Certain changes are especially relevant for their intensity and the importance of the antibiotic family, like the large decreases in resistance observed against ertapenem and fosfomycin among bla_{VIM} ß-lactamases and certain fos genes, respectively; however, the bla_{OXA-48} ß-lactamase from the clinically relevant pOXA-48 plasmid conferred 4-fold higher ertapenem resistance in anaerobiosis. Strong changes in resistance patterns in anaerobiosis were also conserved in *Klebsiella pneumoniae*. Our results suggest that anaerobiosis is a relevant aspect that can affect the action and selective power of antibiotics for specific AMRs in different environments.

A117

Identification and Functional Analysis of the Contribution of the avrA Gene to Vancomycin Resistance in *Enterococcus faecium*

Zhaoxiang Lu[1], Ross McInnes[2], Freya Allen[1], Willem van Schaik[1]

[1]Institute of Microbiology and Infection, Birmingham, United Kingdom. [2]Cancer Research UK Scotland Institute, Edinburgh, United Kingdom

Abstract

The growing challenge of vancomycin-resistant enterococci underscores the need for a comprehensive understanding of theme mechanisms that contribute to vancomycin resistance in the important opportunistic pathogen *Enterococcus faecium*. This study builds on the previous discovery of the *avrA* gene, through Transposon sequencing (Tn-seq) library screening and RNA-seq analysis during growth of *E. faecium* in the presence of vancomycin. The *avrA* gene is proposed to be contributing to the resistance of *Enterococcus faecium* to vancomycin.

In the process of generating a deletion mutant for *avrA* using a novel CRISPR-Cas12a-based approach, we observed the unexpected loss of the vancomycin resistance plasmid. To address this, we employed a Tn-seq library to screen for *avrA* transposon mutants as an alternative strategy to obtain an *avrA* knockout strain. We will then examine the changes in vancomycin susceptibility, growth, and expression of the *vanA* resistance genes in the *avrA* transposon mutant.

Our findings will offer new insights into the molecular foundations of vancomycin resistance in *E. faecium* and could potentially open pathways for the development of novel antimicrobial agents against multidrug-resistant Gram-positive bacteria.

A118

Induction of the *Salmonella* Typhiumurium *O*-antigen Capsule at Sites of Bacterial Persistence

Sana Fairman, Yi Jin

Manchester Institute of Biotechnology, Manchester, United Kingdom

Abstract

The high-molecular-weight *O*-antigen capsule is a bacterial polysaccharide that is required to form *Salmonella* biofilms, allowing environmental desiccation resistance and aggregation on the surface of cholesterol gall stones, the major site of persistent *Salmonella* infection. The sulfoglycolytic- Embden-Meyerhof-Parnas pathway has been implicated in expression of the *Salmonella* O-antigen capsule. Encoded by the *yih* operon, molecular details of the relationship between capsular synthesis and this metabolic pathway for sulfoglycolysis remain to be explored. Here we determine by transcription activity assays, gel shift assays and biofilm formation experiments the activity of potential small molecule effectors regulating the *yih* operon through direct binding of the single target transcription factor *CsqR*. These findings offer a greater understanding of site-specific induction of the *yih* operon at sites of *Salmonella* persistence and indicate potential targets for alleviating the structural persister phenotype. Continued exploration of the transcriptional networks governing bacterial persistence traits is essential for ameliorating the burden of long-term bacterial survival and combatting the growing threat of antimicrobial resistance.

A119

Using experimental evolution to understand antibiotic production in *Streptomyces coelicolor*

Silja Vahtokari[1], John T Munnoch ORCID iD[1], Lorena T Fernández-Martínez ORCID iD[2], Paul A Hoskisson ORCID iD[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom

Abstract

Streptomyces coelicolor is a Gram-positive bacterium, with remarkable potential for natural product biosynthesis. Responsible for production of 2/3rd of clinically relevant antibiotics, understanding how *Streptomyces* regulates antibiotic synthesis and the evolutionary forces that shape this is important in the fight against antimicrobial resistance (AMR). While *Streptomyces* make one or two natural products under laboratory conditions, including antibiotics, their genomes encode the capacity to biosynthesize numerous, from so-called 'silent' biosynthetic gene clusters (BGCs). Developing tools and our understanding to activate these BGC or boost production of known antibiotics in an industrial setting is a key strategy in combating AMR. Using *Streptomyces coelicolor* M1152, a model with major antibiotic BGCs removed, we are studying how epistasis affects antibiotic production using a Long-Term Evolution Experiment (LTEE). The LTEE consists of six distinct, liquid culture lineages, three grown in rich and three in minimal media. These cultures are passaged every three days (1:100 dilution) into fresh media with samples of each passage kept at -80°C for future analysis. Phenotypic characterization and DNA sequencing of both isolates and communities has been carried out at generational milestones. Through quantifying antibiotic production by reintroducing an unmodified actinorhodin BGC, we observe positive and negative epistasis and the appearance of parallel mutations across replicate lines. We are now in the process of linking genotypes to phenotypes while further investigating the cause of epistasis including the putative role of a range of *adpA* alleles. These data highlight the importance of the experimental evolution in understanding antibiotic production and regulation.

A120

An *in-vitro* laboratory evolution model of antimicrobial resistance spread through horizontal gene transfer

Thu Hien Vu[1,2], Marco Galardini ORCID iD[1,2]

[1]Institute for Molecular Bacteriology, TWINCORE Centre for Experimental and Clinical Infection Research, a joint venture between the Hannover Medical School (MHH) and the Helmholtz Centre for Infection Research (HZI), Hannover, Germany. [2]Cluster of Excellence RESIST (EXC 2155), Hannover Medical School (MHH), Hannover, Germany

Abstract

Antibiotic resistance is frequently acquired through horizontal gene transfer (HGT) rather than through mutations inherited vertically. However, the majority of adaptive laboratory evolution (ALE) experiments have focused on single-strain bacterial populations, while communities that are more prevalent in nature have received little attention. Studying evolution through HGT is challenging, as it requires a stable cooperative interaction in a bacterial community. To better study the dynamics of HGT of antimicrobial resistance we are developing an *in vitro* system. In particular, we are developing a synthetic auxotrophic *E. coli* community with fluorescent tags, growing in a culturing device (Chi.Bio), which is capable of tracking a culture in real time. In order to study the spread of last-resort antibiotics, we are using two antimicrobial resistance (AMR) conjugative plasmids, pOXA48 and PN23 (carbapenem and colistin resistance, respectively) as a model for our systems. By running ALE bacterial community experiment, we are investigating community composition changes when a selective pressure is introduced. Through this system, we aim to detect and analyze gene transfer events and observe community dynamics during adaptation to antimicrobials.

A121

Function of RND efflux pumps mediating new tetracycline derivatives resistance in Klebsiella pneumoniae

Qingqing Xu[1,2], Aimaiti Alimire[1]

[1]Institute of Antibiotics, Huashan Hospital, Fudan University, Shanghai, China. [2]Department of Biochemistry, Cambridge, United Kingdom

Abstract

Aim: This study aims to investigate the role of RND efflux pumps AcrAB and OqxAB in new tetracycline derivatives resistance and mechanism of overexpression of *ramA* leading to upregulation of AcrAB in *Klebsiella pneumoniae*.

Methods ： In the present study we investigated function of AcrAB and OqxAB efflux pumps in new tetracycline derivatives resistance in *Klebsiella pneumoniae* by using qRT-PCR, gene knockout and antimicrobial susceptibility testing. PCR products of previously reported tigecycline resistance genes including *ramR* and sRamA5 were sequenced.

Results: Tigecycline-susceptible KP2606 and KP22 which was resistant to tigecycline and eravacycline were collected. KP2606-4 and KP2606-16 were tigecycline-resistant mutants selected by serial passage (4× MIC and of 16× MIC the original strain) in our previous study. We detected overexpression of *ramA* with or without overexpression of *acrAB* in novel tetracycline derivative drugs resistant KP2606-4,KP2606-16,and KP22. Knock out of *ramA* and *acrB* genes led to reduction in tigecycline and eravacycline MIC, while deletion of *oqxB* has no effect on these drug's MIC. Moreover, PCR sequencing showed R108stop of *ramR* mutation in KP22 and C113T of sRamA5 in KP2606-4 and KP2606-16,these mutations could lead to overexpression of *ramA* and upregulation of AcrAB efflux pump mediating novel tetracycline derivative drugs resistance.

Conclusion: Overexpression of RamA-AcrAB is the most potent mechanism mediating novel tetracycline derivative drugs resistance. Role of OqxAB in novel tetracycline derivative drugs resistance seems to be not sufficient. *ramR* and sRamA5 mutation could result in RamA overexpression, further mediating novel tetracycline derivatives resistance in *Klebsiella pneumoniae*.

A122

Prevalence and Cross-Resistance Patterns of Clindamycin-Resistant Group B Streptococcus Strains in Clinical Samples from Najran Hospitals

Abdullateef Alshehri[1], Mohsen Qahtani[2], Osman AE. Elnoubi[1]

[1]Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences, Najran University, Najran, Saudi Arabia. [2]Department of Laboratory Specialist, Specialist Section, Najran Armed Force Hospital, Najran, Saudi Arabia

Abstract

Background: Group B Streptococcus (GBS) poses a significant threat, especially in vulnerable groups like neonates, pregnant women, and immunocompromised patients. Clindamycin-resistant GBS complicates treatment, particularly for patients with penicillin allergies. This study offers an in-depth analysis of clindamycin-resistant GBS isolates from clinical samples in Najran, Saudi Arabia, aiming to understand the prevalence, resistance mechanisms, and implications for local antibiotic stewardship.

Methods: Clinical samples were collected from Najran hospitals, and GBS strains were isolated and tested for antibiotic susceptibility. Clindamycin-resistant isolates underwent comprehensive testing, with minimum inhibitory concentrations (MICs) determined for clindamycin, erythromycin, penicillin, ampicillin, tetracycline, levofloxacin, linezolid, and vancomycin. Interpretations followed guidelines, and descriptive and comparative analyses were applied to assess cross-resistance patterns and potential multidrug-resistant profiles.

Results: Among the clindamycin-resistant isolates, substantial cross-resistance was observed, with over 80% erythromycin-resistant. Approximately 65% of isolates were resistant to tetracycline, indicating multidrug resistance. All isolates remained susceptible to penicillin and ampicillin, reinforcing their reliability for treating GBS. Most isolates were also susceptible to levofloxacin and moxifloxacin, although a small subset displayed intermediate susceptibility to levofloxacin. Linezolid and vancomycin demonstrated full efficacy, suggesting their utility as alternative treatments in cases with multidrug resistance.

Conclusion: This study reveals high rates of cross-resistance to erythromycin and tetracycline in clindamycin-resistant GBS isolates, while penicillin and ampicillin remain effective. The prevalence of multidrug resistance highlights the need for continuous resistance surveillance and tailored treatment protocols. Further investigation into the genetic mechanisms underlying these resistances is essential to improve local antibiotic stewardship and inform evidence-based treatment strategies.

A123

Functional characterisation of a novel MATE transporter from Campylobacter coli

Thamarai K Janganan ORCID iD[1], Bruno Silverster Lopes[2], Vassiliy N Bavro[3]

[1]Middlesex University, London, United Kingdom. [2]Teesside Univesity, Middlesbrough, United Kingdom. [3]Essex University, Colchester, United Kingdom

Abstract

Multi-drug And Toxic compound Extrusion (MATE) family of secondary active transporters are energised by Na^+ and/or H^+ gradients and expel cationic compounds, including several antimicrobials, toxic compounds and dyes. A limited number of studies have been conducted on MATE pumps in Gram-negative bacteria, and particularly none in *Campylobacter* spp*,* which is classified as a serious threat by the CDC and is a significant cause of foodborne infections worldwide.

Here, we describe a novel MATE-transporter from *C. coli* and identify key residues involved in proton translocation and drug binding. *C. coli* MATE (*Cc* MATE) from C606 isolate was cloned and expressed in a heterologous host, *E. coli* KAM32 (DE3). Our study demonstrates that *Cc* MATE is a proton-dependent pump which conferred a 60-fold resistance to erythromycin, novobiocin, and a range of dyes and biocides. Our mutational study revealed a key residue L128, along with a proton-relay network involving residues P14, N29, Y127, N172, and N190 in the N-lobe as critical for proton translocation and also identified the two putative drug binding residues - W243 and R359. While fluoroquinolones are common substrates for most characterised bacterial MATEs, they do not appear to be substrates for *Cc* MATE, indicating that *Cc* MATE may represent a novel transporter, likely a sub-class of the DinF subfamily. The characterisation of this novel transporter improves our understanding of the drug extrusion mechanisms in *Campylobacter* and opens new avenues for therapeutic intervention against drug resistance.

A124

Metagenomic Profiling of Antimicrobial Resistance in Siwa Oasis Therapeutic Hot Springs

Muhammad Ajagbe[1], Ali H. A. Elbehery[2,1], Shimaa F. Ahmed[1], Amged Ouf[1], Basma M. T. Abdoullateef[1], Rehab Abdallah[1], Rania Siam[1,3]

[1]The American University in Cairo, Cairo, Egypt. [2]University of Sadat City, Sadat, Egypt. [3]Ross University School of Medicine, Bridgetown, Barbados

Abstract

Cleopatra and Fatnas Springs in Siwa Oasis, Egypt, are known for their therapeutic properties, attracting visitors seeking natural healing. However, microbial communities in these hot springs may also harbor antimicrobial resistance genes (ARGs) and virulence factors that may pose potential public health concerns. Through high-resolution NovaSeq-6000 shotgun sequencing, we performed a comprehensive metagenomic analysis to characterize microbial diversity, ARG profiles, and virulence genes within these ecosystems.

Taxonomic profiling revealed a rich microbial community dominated by bacteria (~99%) with hardly any archaea (<0.01%). Mesophilic bacteria from the phyla Pseudomonadota, Bacteroidota, Actinomycetota, and Planctomycetota (collectively ~93%) predominated in both springs. We recovered a total of 37 medium-to-high metagenome-assembled genomes (MAGs). Phylogenomic analysis revealed that 50% of the MAGs cluster with genomes from other freshwater sources.

With stringent screening for ARGs, we identified six resistance genes—*efpA*, $bla_{TEM\text{-}116}$, *rbpA*, *aph(3')-Ia*, *mfpA*, and *mmr*—that confer resistance to key antibiotic classes, including aminoglycosides, beta-lactams, fluoroquinolones, isoniazid, and rifamycin. The *efpA* gene was identified in *Mycobacterium ahvazicum* MAG, a non-tuberculous mycobacterium of the *Mycobacterium simiae* complex.

Additionally, we identified 11 virulence factors, with notable genes involved in adhesion, invasion, and immune modulation, underscoring potential pathogenicity. ~50% of these genes were also associated with the *M. ahvazicum* MAG.

Our findings revealed that despite the therapeutic benefits of Cleopatra and Fatnas Springs, they also serve as reservoirs for ARGs and virulence genes. This study underscores the importance of monitoring microbial communities in recreational and therapeutic springs to better understand resistance and pathogenicity in human-exposed environments.

A125

The need for signalling nucleotides to take MRSA to the next step: tuning high-level resistance in a superbug

Laura Carrilero[1], Mark E. Gainford[1], Jeffrey Green[1], Simon J. Foster[1], Rebecca M. Corrigan[2]

[1]University of Sheffield, Sheffield, United Kingdom. [2]University College Dublin, Dublin, Ireland

Abstract

High level methicillin resistant *Staphylococcus aureus* (HL-MRSA) is a global threat, as it causes life-threatening infections. The first step towards gaining low level methicillin resistance is the acquisition of *mecA*, present in the mobile genetic element staphylococcal cassette chromosome *mec* (SCC*mec*). *mecA* encodes PBP2A, a penicillin binding protein with transpeptidase activity and low affinity for β-lactam antibiotics. Acquisition of *mecA* results in an increase in the MIC of oxacillin from 0.25 µg/mL to 2 µg/mL. However, other factors are needed to enable the bacterium to become high-level resistant. These factors, which we call potentiators, are mutations in genes of an eclectic nature. Nevertheless, there are only two potentiators that confer a resistance level above 256 µg/ml when *mecA* is present in the chromosome in single copy: mutations in *rpo* genes B and C *(rpo**) or mutations in *rel* (*rel**). RpoB and RpoC encode for subunits of the RNA polymerase, while Rel is a (p)ppGpp synthetase, with potentiator mutations in Rel likely resulting in increased (p)ppGpp synthesis. Here we explore the role of (p)ppGpp, as well as the two interconnected signalling networks, c-di-AMP and GTP, in the route towards high level resistance in MRSA. We determine the levels of each signalling nucleotide in HL-MRSA, and establish the importance of each system for the resistance phenotype. Our research highlights the importance of signalling nucleotide metabolism and the pangenome on the effects of acquired mobile genetic elements, modulating possible phenotypic landscapes and allowing high-level resistance to occur.

A126

Using random mutagenesis to predict mutations within the KPC-2 gene that confer resistance to β-lactam/β-lactamase inhibitor combinations.

Hawa Ahmed ORCID iD, Maria Rosa Domingo-Sananes, Gareth McVicker, Alasdair Hubbard

Nottingham Trent University, Nottingham, United Kingdom

Abstract

Mutations within β-lactamase genes can alter the substrate and inhibitor specificity of the enzyme, causing resistance to β-lactam/β-lactamase inhibitor combinations (BLICs) to which bacteria were previously susceptible. Many of these mutations have not currently been identified, which can compromise the prediction of antimicrobial resistance (AMR) using genomic data. In this project, we aimed to identify mutations in KPC-2 which confer resistance to ceftazidime-avibactam (CZA) and meropenem-vaborbactam (MEV).

Using a random mutagenesis approach, we generated a mutant library of bla_{KPC-2} in the low-copy plasmid pBR322, screened for resistance to CZA and MEV, and identified the corresponding single nucleotide polymorphisms (SNPs) bioinformatically.

We found that while random mutagenesis produced CZA-resistant mutations within the KPC-2 gene, these mutations did not confer resistance to MEV. The mutational rates and number of SNPs produced varied with each mutagenesis reaction. 58% of the 45 SNPs identified were outside the active site loops. Five were located in the loop between β3 and β4 helix, four in the loop between β5 and α11 helix and 10 in the omega loop, where the clinically relevant D178 mutations were replicated in all the reactions. Only 20% of the SNPs have been reported in clinical isolates. This implies a majority (80%) are novel with the potential to confer resistance to CZA.

Identification of novel SNPs which are involved in resistance to CZA will improve AMR prediction from genomic data. This will directly improve AMR surveillance and effective antibiotic use, thus reducing treatment failure.

A127

Mixtures of non-antibiotic pharmaceuticals and ciprofloxacin can increase selection for antibiotic resistance

April Hayes ORCID iD[1], Lihong Zhang[1], Jason Snape[2], Edward Feil[3], Barbara Kasprzyk-Hordern[3], William Gaze[1], Aimee Murray[1]

[1]University of Exeter, Penryn, United Kingdom. [2]University of York, York, United Kingdom. [3]University of Bath, Bath, United Kingdom

Abstract

Antibiotic resistance is a global threat to human and animal health. There is increasing recognition that environmental pollutants (such as non-antibiotic pharmaceuticals) may select for antibiotic resistance in the environment, which is both a reservoir of, and a sink for, human and animal associated resistance. Quantifying selection for resistance is commonly experimentally evaluated using single compounds. However, this is not realistic for freshwater environments or the human body, where antibiotics will be present alongside non-antibiotic pharmaceuticals such as painkillers. Here, we tested the effects of simple mixtures of one of three non-antibiotic pharmaceuticals (diclofenac, metformin, or 17-β-estradiol) and the antibiotic ciprofloxacin on selection for antibiotic resistance in a complex bacterial community. We found that combinations of non-antibiotic pharmaceuticals and ciprofloxacin reduced community growth more than ciprofloxacin alone. Furthermore, *intI1* qPCR demonstrated that ciprofloxacin was more selective when present in the mixtures, with a reduction in selective concentration from 40µg/L to 10µg/L for all mixtures. Additionally, metagenome sequencing indicated that the mixtures changed the community composition compared to the ciprofloxacin alone treatments, and both positively and negatively selected for antibiotic resistance genes (e.g. *fecE* and *tolC*). These effects often differed between mixture types. Overall, we demonstrate that mixtures of pharmaceuticals can be more selective than each compound individually, but the effects are often subtle and involve specific species and genes. Future research should include mixtures of pollutants to better understand antibiotic resistance gene dynamics in both the clinical and natural environment.

A128

Non-synonymous mutations in penicillin-binding proteins of *Streptococcus pyogenes* reduce susceptibility to beta-lactam antibiotics

Asha L Marshall, Valerie WC Soo

Department of Adult Infectious Disease and Centre for Bacterial Resistance Biology, Imperial College London, London, United Kingdom

Abstract

Streptococcus pyogenes (GAS) is a bacterial pathogen that causes various human infections, ranging from mild pharyngitis to invasive infections, such as sepsis. All GAS isolates are susceptible to beta-lactam (BL) antibiotics. However, GAS isolates with reduced susceptibility to BL antibiotics have recently been reported in the USA, raising the possibility of further evolution of GAS resistance to BL antibiotics. The reduced BL susceptibility has been attributed to non-synonymous mutations in the bacterial penicillin-binding proteins (PBPs); in the UK, little was known whether mutations in *pbp* genes will also lead to reduced susceptibilities to BL antibiotics. In this study, we chose 25 GAS isolates (five *emm1* serotypes and 20 *emm12* serotypes, respectively) and tested their susceptibilities to seven BL antibiotics using Etests and broth microdilution assays. In addition, we verified the presence of non-synonymous mutations in genes *pbp1a*, *pbp2x*, *pbp3.1*, and *pbp3.2* using Sanger sequencing. Compared to reference GAS isolates without *pbp* mutations, the minimum inhibitory concentrations (MICs) of *pbp* mutants to different BL antibiotics increased by 1.3 to 4-fold. In particular, a P601L substitution in PBP2x and an R83K substitution in PBP3.2 led to an approximately 4-fold higher MIC for penicillin and meropenem, as well as a 2-fold higher MIC for cefotaxime, cefuroxime, and amoxicillin. In summary, our results suggest that certain non-synonymous mutations in *pbp* genes will indeed reduce GAS susceptibility to BL antibiotics, and further highlight the need for understanding how these *pbp* mutations may contribute to the evolution of BL resistance in GAS.

A129

Role of the Liverpool Epidemic Strain Prophages on antibiotic resistance of Pseudomonas aeruginosa

Kelsey Broadbent[1], Yueyi Cai[2], Revathy Krishnamurthy[2], Andrew Martin[1], Enrique González-Tortuero[1,2], Joe Latimer[1], Ian B Goodhead[1], Joanne L Fothergill[2], Heather E Allison[2], Chloe E James[1]

[1]University of Salford, Salford, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Pseudomonas aeruginosa has been indicated as a priority organism by the WHO, the pathogen is known for causing chronic lung infections leading to considerable morbidity and mortality in individuals with cystic fibrosis (CF).

The Liverpool Epidemic Strain (LES) has been associated with severe disease and competitiveness in the CF lung linked to the carriage of several active prophages. Previous studies mapped the transcriptomic effects of three LES prophages (specifically Φ2, Φ3 and Φ4), on the well-characterised model host strain PAO1. Differential expression of many lysogen host genes was observed compared to the naïve strain. Of note, this included several pathways involved in biofilm formation and other antimicrobial resistance mechanisms (e.g., efflux pump expression).

This study aims to further investigate the effects of LES prophage carriage on biofilm formation and antimicrobial resistance of *P. aeruginosa* hosts using a several phenotypic assays to identify how such prophages enhance the fitness of their host cells in the CF lung. The static crystal violet biofilm assay showed remarkable increases in PAO1 biofilm density when carrying prophage 3. This was affected by media composition and further increased with the dual carriage of prophages 2 and 3. The Kirby-Bauer assay indicated slight differences in antibiotic susceptibility in lysogens compared to naïve PAO1, but all were considerably more susceptible than the original clinical prophage host LESB58.

IMPORTANCE: Preliminary data shows LES phages affect biofilm density of their host through specific interactions. Further investigation in clinically relevant conditions could identify unique targets for tackling recalcitrant *P. aeruginosa* infections.

A130

In vitro evolution of *Enterococcus faecium* in the presence of vancomycin

Freya Allen[1], Ross McInnes[2], Willem van Schaik[1]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Cancer Research UK Scotland Institute, Glasgow, United Kingdom

Abstract

The Gram-positive bacterium *Enterococcus faecium* is an important cause of nosocomial infections. There are limited treatment options for *E. faecium* infections, particularly when acquired transposons confer vancomycin resistance. To identify whether *E. faecium* can evolve additional mechanisms to resist vancomycin, the vancomycin-susceptible *E. faecium* strains E1162 and E2560 were evolved *in vitro* in increasing concentrations of vancomycin for 11 d. We consistently observed evolved populations growing at increased concentrations of vancomycin (from 0.5 µg/ml to 1 µg/ml vancomycin). Genome sequencing of evolved strains revealed single nucleotide polymorphisms (SNPs) in genes encoding the WalKR/YycFG two-component system (TCS), which is involved in regulation of peptidoglycan biosynthesis in Gram-positive bacteria. Both E1162 and E2560 had evolved replicates exhibit non-synonymous SNPs in *walK*, which encodes the histidine kinase in the WalKR TCS. A further E1162 evolved replicate had a SNP in *yycI*, which encodes a protein predicted to regulate WalK activity. Morphological changes were observed in evolved strains in comparison to parent strains, particularly an increase in chain length and a significant increase in cell wall thickness which may be responsible for the decrease in susceptibility to vancomycin. Characterisation of the mutations in the evolved strains is being conducted by RNA-seq and ChIP-seq of WalR is being conducted to identify genes which are regulated by the TCS in *E. faecium.*

A131

An *In Vivo* Infection Model for the Role of a Rtc RNA Repair in Bacterial Virulence and Antibiotic Resistance

Wenqian Zhang, Ahira Varkey, Danai Irakleidi, Martin Buck, Ioly Kotta-Loizou

Imperial College London, London, United Kingdom

Abstract

The RNA 3'-terminal phosphate cyclase (Rtc) system is a conserved bacterial RNA repair system, consisting of the RNA cyclase RtcA, the RNA ligase RtcB, and the transcriptional activator RtcR. Previous studies indicated that the Rtc system is linked to bacterial proliferation and its activation can be induced by ribosomal stressors, including certain antibiotics. This raises the question of whether the Rtc system influences bacterial pathogenicity *in vivo* and contributes to intrinsic resistance against antibiotics. This study developed an *in vivo* infection model using *Escherichia coli* and *Galleria mellonella* to investigate the role of the Rtc system in bacterial pathogenicity and resistance against ribosome-targeting antibiotics. Wild type *E. coli* and strains lacking Rtc components were used to infect *G. mellonella* larvae under both non-stress conditions and in the presence of chloramphenicol or tetracycline. Bacterial pathogenicity was evaluated through larval survival rates and health indices. Results demonstrated that *E.col*i strains with fully functional Rtc systems displayed significantly higher virulence than those with Rtc defects. Antibiotic treatments revealed that a functional Rtc system can enhance bacterial tolerance to these drugs *in vivo*. Pathogenicity assays using blue-fluorescent *E. coli* strains are currently being conducted to study *in vivo* bacterial localisation in order to further explore the reasons underlying the observed differences in virulence. This study contributes to understanding the link between the Rtc RNA repair system and antibiotic tolerance, emphasising its role in bacterial virulence, and providing insights into addressing the growing challenge of antimicrobial resistance.

A132

Uncovering Alternative Mechanisms of Biocide Tolerance in *Proteus mirabilis*

Maryam Y. Ravari, Vicky Bennett, Brian Jones

University of Bath, Bath, United Kingdom

Abstract

The rapid growth of antibiotic resistance has led to reliance on biocides to control infections. However, concerns are rising over bacteria evolving to tolerate concentrations higher than those currently used in biocidal products. Uropathogen *Proteus mirabilis (P. mirabilis)* often exhibits high tolerance to chlorhexidine (CHD), a biocide used clinically. Our previous studies identified several mechanisms of biocide tolerance in *P. mirabilis,* including SmvA efflux upregulation via truncation of *smvR*, and truncation of *mipA* involved in cell wall structure. However, some clinical isolates displaying the highest levels of biocide tolerance do not have these key mutations, indicating that other mechanisms are also involved. To investigate mechanisms in one such isolate, RS42, we used transposon mutagenesis to screen for mutants with increased CHD susceptibility. We identified an RS42 mutant with defective LPS biosynthesis (RS42A) being more susceptible to CHD and other biocides. This is similar to our findings in other clinical isolates that have indicated the importance of LPS structure in biocide tolerance. We also utilised RNA-seq to compare expression changes between high and low-tolerance strains. We identified that the expression of *tet(J),* encoding a tetracycline efflux pump, was significantly upregulated in RS42 compared to low-tolerance and other high-tolerance isolates. Genomic analysis showed a mutation causing truncation of the corresponding *tetR* repressor, indicating potential constitutive upregulation of the efflux system. Overall, these findings contribute to our understanding of biocide adaptation and tolerance mechanisms in this pathogen.

A133

The impact of triclosan on antibiotic tolerance in Staphylococcus aureus through stringent response pathway.

Parvati Iyer ORCID iD[1,2], Dean Walsh[3], Andrea Salzer[2], Christiane Wolz[2], Kim Hardie[1], Jonathan Aylott[1]

[1]University of Nottingham, Nottinghamshire, United Kingdom. [2]University of Tuebingen, Tuebingen, Germany. [3]Queen Mary University, London, United Kingdom

Abstract

The biocide triclosan is used extensively in both household and hospital settings. The chronic exposure to the biocide occurring in individuals that use triclosan-containing products results in low levels of triclosan present in the human body. Triclosan was proposed to induce antimicrobial resistance in bacteria (Suller & Russell, 2000). Antimicrobials have failed to control *Staphylococcus aureus* and often infections persist or relapse. Stringent response mediated by alarmones ppGpp and (p)ppGpp have been reported to induce various virulence pathways and might be involved in antibiotic resistance and tolerance (Salzer & Wolz, 2023). Here, we aim to analyse whether the fatty acid inhibitor triclosan impacts antibiotic tolerance in planktonic and biofilm grown *S. aureus*. We analysed different *S. aureus* strains and mutants deficient in (p)ppGpp synthesis. We show that physiological concentrations of triclosan protects *S. aureus* from bacterial killing by ciprofloxacin and vancomycin. Triclosan pretreatment also protected *S. aureus* biofilms against antibiotics as shown by live/dead cell staining and viable cell counting (Walsh, Salzer, Wolz, Aylott, & Hardie, 2023). In planktonic cultures the triclosan effect on antibiotic tolerance was independent of (p)ppGpp and there was no induction of the stringent response by triclosan treatment. However, in biofilms antibiotic tolerance was decreased in a $pppGpp^0$ mutant. This suggests that the mode of action of triclosan varies in planktonic and biofilm. RNA-sequencing is unravelling the molecular mechanism of triclosan induced tolerance which is further confirmed by metabolomic studies.

A134

Development Of A Polymicrobial Aggregate Biofilm Model For Use In Preclinical Testing Of Novel Cystic Fibrosis Therapeutics

Hollie Leighton ORCID iD[1], Dilip Nazareth[2], Aras Kadioglu[1], Daniel Neill[3], Joanne Fothergill[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Liverpool Heart and Chest NHS Foundation Trust, Liverpool, United Kingdom. [3]University of Dundee, Dundee, United Kingdom

Abstract

In the lungs of individuals with cystic fibrosis (CF), microbes colonize and form biofilms that contain multiple species of bacteria and fungi. Interactions between these organisms are often overlooked when evaluating the effectiveness of antimicrobial treatments. Additionally, the lung environment in CF is altered, with CF sputum containing high levels of mucin, extracellular DNA, and amino acids. This altered environment can also impact the resistance of bacteria within this niche.

A polymicrobial biofilm model was developed using *Pseudomonas aeruginosa* LESB58, *Staphylococcus aureus* and *Candida albicans* in synthetic sputum, across various oxygen concentrations to mimic lower oxygen levels in the centre of biofilms, and within microaerophilic pockets in the lungs. Anti-biofilm agents such as DNase were also trialled in the model to observe the effects on the resistance of the biofilms against two clinical antibiotics.

Different types of microscopies, such as fluorescence microscopy and cryo-scanning electron microscopy, were employed to investigate the architecture of biofilms further. Additionally, proteome data analysis was conducted to identify specific proteins whose abundance levels fluctuate in response to varying environmental conditions, ultimately contributing to the biofilms' resistance against antimicrobial agents and enhancing their pathogenicity. One such protein, PvdF, involved in iron acquisition and pyoverdine production, was found to be more abundant in polymicrobial biofilms compared to mono-species biofilms, highlighting its role in multispecies dynamics.

By integrating these methodologies, the study aimed to deepen the understanding of the mechanisms underlying biofilm behaviour and resilience, and provide a relevant platform for testing novel therapeutics under more realistic conditions.

A135

Investigating single-cell mechanisms of bacterial antibiotic persistence using high-throughput microfluidics-microscopy and fluorescent genetically encoded physiology reporters

Kieran Abbott ORCID iD, Erez Li ORCID iD, Georgeos Hardo ORCID iD, Somenath Bakshi ORCID iD, Ashraf Zarkan ORCID iD

University of Cambridge, Cambridge, United Kingdom

Abstract

Bacterial antibiotic persistence is a phenotypic mechanism of antibiotic resistance by which a small subpopulation of cells survive antibiotic exposure through transient dormancy or semi-dormancy. These persisters have been associated with a range of recurrent and chronic infections, and raise several questions: What are the physiological signatures of persistence? Do these signatures result from antibiotic treatment, or are they present prior to antibiotic exposure? Are there universal signatures of persistence, or do they depend on the mechanism of antibiotic action or environmental conditions?

As persisters are phenotypic outliers within a population, single-cell level phenotypic analysis is essential to identify these underlying mechanisms. However, single-cell level analysis has proven to be difficult, due to the rarity and transient nature of persistence. Therefore, we utilise custom-designed high-throughput microfluidic devices with time-lapse microscopy to study up to 100,000s of single cells in parallel, allowing for identification and quantification of these rare and transient events. In combination with a suite of optimised genetically encoded fluorescent reporters in E. coli for key aspects of bacterial physiology, we investigate the physiological signatures of persister cells before, during, and after antibiotic treatment – using different antibiotics and growth conditions to identify patterns in the physiological signatures of antibiotic persistence.

These key physiological signatures could be utilised to develop highly parallel screening of persister formation, for purposes such as screening novel anti-persister therapeutics, or for further investigating the state of persister cells prior to antibiotic treatment, such as through single-cell transcriptomics.

A136

Tracking AMR in a Changing Climate: A Comparative Analysis of Resistance Genes in Flooded and Non-Flooded Soils.

Eva Kilcoyne[1], Shauna O'Shea[1], Juan Ignacio Vilchez[2], Ruben Rosencrantz[3], Fiona Walsh[1]

[1]Maynooth University, Maynooth, Ireland. [2]ITQB NOVA, Lisbon, Portugal. [3]Fraunhofer-Institut für Angewandte Polymerforschung, Potsdam, Germany

Abstract

Antimicrobial resistance (AMR) and climate change are among two increasing threats to global health. As a result of climate change, increased flooding events have been recorded across the globe. The environment, including water bodies, soil, and air can act as reservoirs for antimicrobial resistance genes (ARGs). These genes can spread among microbial communities and be transferred to pathogenic bacteria through methods such as horizontal gene transfer. Surveillance is needed to identify and monitor these reservoirs to understand how they contribute to the broader spread of AMR.

This study investigates the surveillance of AMR in the context of environmental changes, comparing methods to evaluate resistance gene prevalence and diversity in both flooded and non-flooded soils. Eight site matched flooded and non-flooded soils were sampled across Ireland. DNA was extracted from all samples and extensive quality control procedures were performed. Shotgun metagenomic sequencing was performed on the DNA, followed by in-depth bioinformatic analysis to reveal the resistance gene profiles of the flooded and non-flooded soils. The results showed an increase in antimicrobial resistance genes (ARGs), including *qacG* and *vanH* (within the *vanA* and *vanB* clusters) in flooded soils. To further assess this, quantitative PCR (SYBR Green) will be used to detect and quantify critical priority resistance genes in both flooded and non-flooded soils. These target genes include $bla_{CTX\text{-}M}$, bla_{TEM}, bla_{SHV}, bla_{KPC}, bla_{NDM}, bla_{OXA}, bla_{VIM}, and bla_{IMP}. Additionally, a comparison will be conducted between the results of the two methods and determine their effectiveness for AMR surveillance in soils.

A137

Evolution of antimicrobial resistance in bacterial microbiomes

Yige Sima[1], Issy Garratt[1], Matthew Wand[2], Tiffany Taylor[1], Mark Sutton[2], Eshwar Mahenthiralingam[3], Brian Jones[1]

[1]University of Bath, BATH, United Kingdom. [2]UK HSA, Salisbury, United Kingdom. [3]Cardiff University, Cardiff, United Kingdom

Abstract

Antimicrobial resistance (AMR) is one of the most pressing challenges facing humanity, with a current burden of 5 million deaths per year (to rise to an estimated 50 million per year in 2050). Increased use of biocides such as chlorhexidine have become common features of enhanced infection control practices that aim to reduce antibiotic use and limit the spread of resistance. However, there is accumulating evidence that bacterial pathogens can adapt to become less susceptible to biocides, and in some cases, this has been linked to cross-resistance to antibiotics. Despite this, the underlying mechanisms leading to acquisition of reduced biocide susceptibility are not well characterised overall, and it remains unclear how acquisition of biocide tolerance influences fitness in the polymicrobial communities bacteria form in a range of settings. Here we aim to apply random transposon mutagenesis in conjunction with a novel polymicrobial model of catheter associated urinary tract infection (CAUTI) to elucidate mechanisms underpinning acquisition of biocide tolerance in *Klebsiella pneumoniae* and evaluate how these impact survival in a polymicrobial CAUTI community. A library of mini-Tn5 *K. pneumoniae* mutants will be screened to identify those with reduced susceptibility to chlorhexidine. Mutants will be characterised to identify disrupted genes, assess polar effects of Tn insertion, and evaluate a range of relevant phenotypes including growth, biofilm formation and susceptibility to antibiotics. Polymicrobial models of CAUTI will be used to assess the ability of mutants and the WT parental strain to persist within microbial communities.

A138

Evidence for clonal expansion and plasmid-mediated horizontal gene transfer as mechanisms for the dissemination of Extended-Spectrum Beta-Lactamases producing Escherichia coli in poultry in Harare, Zimbabwe

Alistair Davies ORCID iD[1], Peter Katsande[2], Thomas Chisnall[1], Kudzaishe Vhoko-Tapesana[3], Sam Willcocks[4,5], Chenai S Majuru[2], Tendayi Mubau[6], Richard Stabler[4], Roderick M Card[1]

[1]The Animal and Plant Health Agency, Weybridge, United Kingdom. [2]Department of Veterinary Technical Services, Harare, Zimbabwe. [3]Food and Agriculture Organization of the United Nations, Harare, Zimbabwe. [4]Department of Infection Biology, London, United Kingdom. [5]Department of Biosciences, London, United Kingdom. [6]Masvingo Provincial Veterinary Laboratory, Masvingo, Zimbabwe

Abstract

Extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli are resistant to the critically important third and fourth generation cephalosporin antibiotics and present a* risk to animal and human health. In Zimbabwe there is an evidence gap concerning the prevalence and diversity of ESBL-producing *E. coli* in poultry. In this study we screened for ESBL-producing *E. coli* at farms (n=50) and markets (n=10), according to ISO standards. Seventy ESBL-producing E. coli were obtained and examined by antimicrobial susceptibility testing and long-read & short-read genomic sequencing. Geographic Information System mapping was used to visualise the distribution of the ESBL-producing clones.

Genomic analysis revealed clonal groups of isolates and provided evidence for clonal expansion and horizontal gene transfer via plasmids being responsible for the dissemination of ESBL-producing *E. coli*. For example, isolates of ST1141 harbouring a 330kbp IncHI2 plasmid with $bla_{\text{CTX-M-15}}$ were present at multiple sites. In contrast, a ~210kbp IncHI2 plasmid that carried $bla_{\text{CTX-M-14}}$ was present in isolates from five different STs which were isolated from multiple sites. A total of eight distinct $bla_{\text{CTX-M}}$ gene variants were identified in the isolate panel and all were located on plasmids. All isolates were multidrug resistant.

This study underscores the importance of AMR surveillance in poultry as part of Zimbabwe's national action plan. Findings highlight the need for One Health approaches, such as improved biosecurity and WASH measures, to limit the spread of AMR genes, and reinforce the call for policies on appropriate antibiotic use in humans and agriculture to reduce AMR transmission risks to humans.

A139

Membrane transporters mediate the bactericidal action of Colistin in *Escherichia coli*

J. Enrique Salcedo-Sora ORCID iD, Rhysq Donafee

University of Liverpool, Liverpool, United Kingdom

Abstract

Colistin is a bactericidal lipoprotein used as a last line antibiotic against Gram-negative bacteria infections. As with other polycations it kills bacteria by disrupting the bacterial cell membranes after initial electrostatic interactions with the outer membrane lipopolysacharides (LPS). Concomitantly it displaces cations from the LPS. Altogether, this class of antibiotic causes cell death by disregulation of the cell permeability. It seemed then logical to query the potential mediation of membrane transporters, integral cell membrane proteins, in the toxic effects of bactericidal lipoproteins. After screening 534 gene knock out mutants in *E. coli* for tolerance to Colistin, strains lacking *clcB*, *ptsI* and *ycaM* had several-fold increased tolerance to this antibiotic. On the other hand, strains over-expressing *clcB*, *ptsI* and *ycaM* sensitised *E. coli* to Colistin. clcB is a proton/chloride antiporter, ptsI is a cytoplasmic protein part of the phosphoenolpyruvate:sugar phosphotransferase permease system, and ycaM is a putative amino acid-polyamine-organocation antiporter. We present further experimental evidence and animal survival assays using the greater wax moth (*Galleria mellonella*) larvae model, that substantiate the case for an active role of membrane transporters/transport systems in the bactericidal action of lipoproteins such as Colistin. Given the evidence presented here the rapid killing caused by the membrane permeability effects of Colistin should be rationalised taken into account the specific cell disruptions caused by disregulated membrane transporters/transport systems.

A140

Why are antibiotic resistance genes mobile?

Eliza Rayner ORCID iD, Daniel Cazares Lopez, Craig Maclean

University of Oxford, Oxford, United Kingdom

Abstract

Bacterial pathogens frequently become resistant to front-line antibiotics by acquiring conjugative plasmids, self-transmissible molecules of DNA that unrelated bacteria can exchange with one another. The stable existence of plasmids is an evolutionary paradox, and the extent to which conjugation aids the persistence of antibiotic resistance plasmids despite this paradox is not fully understood.

Firstly, I used experimental evolution to test the evolutionary benefits of conjugation under varying levels of immigration by plasmid-free recipient cells. I found that higher conjugation rates did not lead to increased plasmid prevalence even at high levels of immigration. Secondly, I tested the effect of predation by a plasmid-dependent bacteriophage on the prevalence of conjugative antibiotic resistance plasmids. While the plasmid-dependent bacteriophage reduced conjugation and caused an initial decline in plasmid prevalence, plasmid prevalence recovered after 12 days serial passage due to vertical plasmid transmission.

These results question the long-term benefits of parasitic plasmid transmission strategies involving very high conjugation rates at the host's expense, and it remains unclear where the adaptive value of conjugation systems lies. Further exploration of this question could improve understanding of why conjugative plasmids play such an important role in the antibiotic resistance crisis and limit the further spread of antibiotic resistance genes by conjugation.

A141

Investigating AMR Dynamics in Complex Environments

Onalenna Neo ORCID iD, Adrian Cazares ORCID iD, Nicholas Thomson ORCID iD

Wellcome Sanger Institute, Cambridge, United Kingdom

Abstract

Horizontal gene transfer (HGT) is a primary driver in the spread of antimicrobial resistance (AMR), with antimicrobial resistance genes (ARGs) often mobilized via vectors such as plasmids and transposons. However, the dynamics of these vectors and their bacterial hosts in different environments remain poorly understood. This study will explore ARG vector dynamics in samples collected longitudinally from water samples. Coliform bacteria will be isolated on Brilliance coliform agar, and colonies sequenced using Oxford Nanopore long-read technology. Genomic sequences and plasmids will be reconstructed, and ARGs identified. Sequences will be screened against a comprehensive database of plasmids to determine if ARG-containing plasmids matches previously catalogued plasmids. The association of transposons and other mobile genetic elements (MGEs) with ARGs, will be investigated. Temporal analysis will be performed to explore distinct patterns in AMR-vector dynamics, where some of the ARG-vector pairs could persist across sampling intervals or display vector versatility. Understanding these ARG-vector dynamics is crucial for assessing bacterial adaptation, resistance evolution, and potential public health risks associated with ARGs across environments.

A142

Can antibiotic combination therapy limit the spread of antibiotic resistance?

Louise Flanagan, Magdalena Kurteu, Danna Gifford, Jessica Forsyth

University of Manchester, Manchester, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a major threat to global health in the fight against bacterial infections and disease. AMR is a particular issue when treating urinary tract infections (UTIs), where resistance to many first-line antibiotics is common. One proposed approach to limit the spread of AMR is combination therapy, using two antibiotics simultaneously to treat an infection.

Here we evolve two strains of E. coli (wildtype and mutator) against gradually increasing concentrations of antibiotic combinations –nitrofurantoin, nitroxoline, cycloserine, ciprofloxacin– each being a commonly used antibiotic to treat UTIs. Since each antibiotic targets different cellular functions it is presumed that multiple mutations will be required to gain resistance, which should constrain its emergence.

We found that a combination of nitroxoline and cycloserine was most effective at suppressing resistance across both wildtype and mutator strains. We also found that there was a much higher incidence of resistance emergence in the mutator strain compared to the wildtype strain. This work may help inform clinical decisions when treating UTIs, and provide insights into the effectiveness of combination therapies

A143

Plasmid-Mediated Fitness and Phenotypic Variation in Klebsiella pneumoniae ST2096 under Antibiotic Stress

Raneem Hirayban, Mathew Milner, Danesh Moradigaravand

KAUST, Thuwal, Saudi Arabia

Abstract

Klebsiella pneumoniae is a multidrug-resistant opportunistic pathogen whose resistance is often facilitated by mobile plasmids carrying both resistance and virulence genes. In Saudi Arabia, sequence type ST2096 is a prominent lineage, which has been recently reported, yet its phenotypic characteristics remain uncharacterized. This study examines the fitness of clinical ST2096 isolates with plasmids carrying extended-spectrum β-lactamases and/or carbapenemases and compares it to other global circulating strains. Growth was assessed over 24 hours under standard or antibiotic stress conditions. Our results indicate notable phenotypic variation linked to plasmid variation, genetic makeup, and infection source. Isolates harboring multiple resistance and virulence genes exhibited a synergistic growth advantage under high antibiotic pressure, highlighting the intricate relationship between genotype, plasmid variation, and phenotype in *K. pneumoniae*.

A144

Generation of a Novel Vancomycin Resistant *Staphylococcus aureus* Strain via Filter Mating for Use in Proteomic Analysis

Gavin Dillon ORCID iD, Fiona Walsh

Maynooth, Maynooth, Ireland

Abstract

Background

Staphylococcus aureus is a gram-positive bacteria found on human skin and is most often associated with skin infections such as abscesses and cellulitis. *S. aureus* has emerged as a worldwide nosocomial pathogen acquiring resistance to antibiotics such as methicillin (MRSA) and vancomycin (VRSA). New methods of treating antibiotic-resistant infections must be developed as it is predicted that by 2050, global deaths related to antibiotic-resistant infections could rise to 10 million annually. While cases of VRSA infections primarily occur in India and the United States, understanding how the VRSA proteome functions will prevent further spreading.

Methods

Grow vancomycin-resistant *Enterococcus faecium,* which carries a plasmid-mediated *vanA* gene, and S. *aureus* strain RN4220, which is vancomycin susceptible, overnight at 37C in BHI broth.

Centrifuge 1mL of each culture, resuspend both pellets in the same 1mL of LB broth and pipette 100uL of this mixture onto a nitrocellulose membrane placed on an LB agar plate. Incubate ON at 37C.

Wash membrane with 10% NaCl, plate the resuspension onto MSA plates supplemented with vancomycin and incubate ON at 28C. Screen colonies for *vanA* gene using PCR.

Results/Discussion

Using this method a RN4220 strain carrying the *vanA* gene was generated. The Minimum Inhibitory Concentration (MIC) of Vancomycin for this new strain was found via micro-broth dilution and it exceeded the breakpoint for what would be considered resistant for *S. aureus*, according to EUCAST guidelines.

Conclusion

This method is a simple way of creating a VRSA strain which can be used in further study.

A145

Unraveling the Secrets of *Klebsiella pneumoniae*: A Combined Machine Learning and Systems Biology Approach to Characterize and Predict the Phenotypic Profile of Clinical Strains

Mathew Milner[1], Jiayi Huang[1], Ge Zhou[1], Raneem Hirayban[1], Manuel Banzhaf[2], Danesh Moradigaravand[1]

[1]King Abdullah University of Science and Technology, Thuwal, Saudi Arabia. [2]University of Newcastle, Newcastle, United Kingdom

Abstract

Klebsiella pneumoniae is an opportunistic gram-negative human pathogen increasingly recognized as a healthcare burden globally. It causes a wide range of acute and chronic infections that are challenging to treat due to its capacity to withstand and adapt to stressors, ultimately enabling multidrug resistance in clinical settings. *K. pneumoniae* harbours a dynamic genome repertoire underlying diverse adaptive mechanisms for resistance, virulence and adaptation in clinical settings. Thus far, the genotype-phenotype map of *K. pneumoniae* has been poorly characterized in clinical strains.

We provided a holistic and comprehensive phenome-genome map in a diverse large-scale panel of clinical *K. pneumoniae* isolates. We employed whole genome sequencing of a diverse collection of *K. pneumoniae* multidrug resistant and virulent isolates (>1500 strains), with a large-scale phenotypic screen consisting of ~150 antimicrobial and environmental stresses. To integrate the genomic and phenomic (stress-response) data, we used a toolkit of interpretable machine learning platforms, including recurrent neural networks, ensemble models, and regularized regression models. The models not only allowed for the precise prediction of antimicrobial resistance from genomic data but also provided a mechanistic understanding of resistance through the identification of robust, reproducible, and predictive biomarkers for different stress conditions.

We are currently planning to deploy our model as a package and web portal for the genome-based rapid characterization of clinical *K. pneumoniae* strains.

A146

When resistance becomes stealthy: characterisation of a novel KPC-2 carbapenemase variant exhibiting antimicrobial resistance gene silencing and ceftazidime-avibactam resistance.

Milli Wilde, Hishikha Reesaul, Alasdair T. M. Hubbard

Nottingham Trent University, Nottingham, United Kingdom

Abstract

In the face of the continued rise of antimicrobial resistance (AMR), predicted to rise to 8.22 million deaths globally by 2050, examining the factors affecting AMR expression is critical. One such factor, AMR gene silencing, has been shown to complicate the selection of the correct treatment of infections; where an AMR gene is predicted to be present from genomic data, but a mutation silences phenotypic resistance.

In this study, we identified a novel KPC-2 variant, *bla*$_{KPC-84}$ which contains a single nucleotide polymorphism (SNP) resulting in the T242P mutation. We characterised the effect of this mutation on fitness, beta-lactamase enzyme and antimicrobial susceptibility in clinical uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* isolates.

Here, we found that the T242P mutation silences resistance to all carbapenemases tested, appearing phenotypically susceptible. Instead, this mutation conferred resistance to the beta-lactam/beta-lactamase inhibitor combination, ceftazidime-avibactam. We found that the effect of the mutation on fitness was diverse and dependent on both strain and species. Interestingly, the T242P mutation significantly reduced the beta-lactamase activity as assessed by nitrocefin hydrolysis. Finally, we assessed the frequency of the mutation that could arise within a population following selection by ceftazidime-avibactam.

This study highlights the need to fully explore the impact of silencing of antimicrobial resistance by mutation. SNPs within important genes which silence known resistance mechanisms while also altering the resistance profile will have implications for the prediction of AMR from genomic data.

A147

Advancing Metagenomic Approaches for AMR Surveillance in Australian Wastewater

Pooja Lakhey ORCID iD[1,2], Naixiang Zhai[1], Anne Leonard[2], Aimee Murray[2], Kevin Thomas[1], Will Gaze[2], Jake O'Brien[1]

[1]The University of Queensland, Brisbane, Australia. [2]University of Exeter, Cornwall, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a growing global health crisis, projected to become the leading cause of death by 2050. However, finding representative data on AMR is difficult. To address this, wastewater based epidemiology has emerged as a critical field, as tracking AMR in wastewater provides essential insights into both regional and global dissemination patterns. While existing studies often focus on prevalent pathogens and resistance genes in urban and accessible areas, this study expands the scope by covering 32 sampling sites across Australia. These data can be standardised and shared globally, enhancing international collaboration and comparison. We analysed wastewater over two survey periods, employing metagenomic sequencing to identify antibiotic-resistant bacteria (ARB), resistance genes (ARGs), and mobile genetic elements (MGEs). The methodology includes DNA extraction, construction of DNA libraries, and sequencing, followed by robust bioinformatic analyses using Fastp for quality filtering, MEGAHIT for contig assembly, and tools like Kraken 2, Bracken, ARGs-OAP 3.0, RGI, and Blast v2.15.0 against the SARG and CARD databases for detailed annotation. Our findings highlight a concerning prevalence of priority pathogens like *Acinetobacter baumannii, Escherichia coli* and *Klebsiella pneumoniae,* with sporadic occurrences of *Mycobacterium tuberculosis* and *Neisseria meningitidis*. Notably, there was widespread identification of genes resistant to critical antibiotic classes, including Macrolides and β-Lactams. The risk assessment of ARGs based on their mobility and pathogenic potential revealed an alarming abundance of high-risk ARGs associated with MGEs, underscoring the urgent need for continued surveillance.

A148

Remodelling the stoichiometry of ParDE toxin-antotoxin systems from *Mycobacterium tuberculosis* to understand DNA gyrase poisoning by ParE

Matthew Grobbelaar ORCID iD, Tim Blower

Durham University, Durham, United Kingdom

Abstract

Type II toxin-antitoxin systems consist of a proteinaceous toxin and antitoxin pair. It has recently been shown that changes in the toxin:antitoxin stoichiometry within these complexes can be thermally driven. Thermally remodelling these complexes leads to the release of the toxic components of these complexes which when liberated can inhibit essential cellular processes. *In vivo* toxin release is utilised by bacteria for plasmid maintenance within a population as well as to inhibit growth in unfavourable growth conditions. Translationally, thermally driven toxin release can be exploited in drug development. In this study, we show that the ParDE1 and ParDE2 toxin-antitoxin systems in *M. tuberculosis* can be remodelled to release the toxic proteins ParE1 and ParE2, respectively. This toxin liberation leads to DNA gyrase inhibition and the arrest of transcription and DNA replication. Using cryo-EM, we have solved the structures of a 217 base pair DNA fragment chirally wrapped around DNA gyrase poisoned by both ParE1 and ParE2. These structures give insight into the binding domains of ParE1, ParE2 and DNA gyrase as well as the active amino acid residues in the ParE toxins.

A149

Stronger together: association between multi-drug resistance (MDR) and biofilm forming ability of Salmonella spp. in poultry processing environments

Mona Abdelmaksoud ORCID iD[1], Muiz Akinyemi ORCID iD[2], Ronald Dixon[1], Bukola Onarinde ORCID iD[1]

[1]University of Lincoln, Lincoln, United Kingdom. [2]University of Leeds, Leeds, United Kingdom

Abstract

Salmonella spp. pose a significant challenge to food safety particularly within the poultry industry. Since *Salmonella* persists in poultry processing plants by forming biofilms, this study investigated conditions for their formation and whether it is correlated with their MDR status. A total of 54 *Salmonella* isolates previously recovered from poultry processing plants were tested at 4°C, 12°C, 25°C, and 37°C using the microtiter plate assay. Biofilm formation strength varied by temperature: at 4°C, 28 isolates produced weak biofilms and 26 moderate; at 12°C, 4 were non-producers, 20 were weak, 28 were moderate, and 2 were strong producers. At 25°C, 23 were weak, 28 moderate, and 3 strong, while at 37°C, more showed strong biofilm production (23 weak, 11 moderate, 20 strong). Statistical analysis significance of temperature ($F(3,432) = 49075.424$, $p < 0.001$, $\eta^2 = 0.997$), isolate ($F(53,432) = 18081.669$, $p < 0.001$, $\eta^2 = 1.000$), and their interaction ($F(159,432) = 1808.328$, $p < 0.001$, $\eta^2 = 0.998$) on biofilm formation. An inverse relationship between MDR status and biofilm strength ($p < 0.001$) was shown and clearly non-MDR strains formed stronger biofilms than MDR isolates. Although WGS analysis of the 5/54 isolates tested identified : *csgD*, *fimA*, *sadA*, *lpfA*, *bssS* as adherence genes, their role in biofilm formation remains uncertain. These findings underscore the impact of temperature and MDR status on biofilm production in *Salmonella*, highlighting that non-MDR strains might depend more on the protection provided by the biofilm community to survive in processing environments.

A150

Investigating the interplay between the evolution of antimicrobial resistance and fungicide use in soil bacterial populations.

James McCartney, Siobhan O'Brien

Trinity College Dublin, Dublin, Ireland

Abstract

The evolution of antimicrobial resistance among bacterial populations has cemented itself as a major global health concern with a projected 10 million individuals to die per year by 2050 as a result. The emergence of antimicrobial resistance is often associated with the misuse of antimicrobials in a clinical setting. However, similar evolutionary trends have been observed in an agricultural environment. This can be resultant of 'priming', whereby an organism's survival capability under stress is enhanced through prior exposure to a milder stress event. The application of selective agents such as pesticides, fungicides, and antibiotics, can drive the evolution of cross-resistance in soil-dwelling bacterial populations. Such a phenomenon was observed in fungicide-treated populations of *Pseudomonas fluorescens* SBW25.

This study investigates how the length of exposure to fungicides impacts the priming of bacterial populations for survival in the presence of other stressors, such as antibiotics like tetracycline and nalidixic acid. Twelve genetically homogenous populations of *Pseudomonas fluorescens* SBW25::Gent^{R} were grown in six sets of semi-natural soil microcosms while exposed to sub-lethal concentrations of the fungicide Fubol Gold for varying periods of time. This was followed by six weeks of exposure to both tetracycline and nalidixic acid.

To this effect, we hypothesize that long-term application of Fubol Gold elicits a stronger priming response in populations of *Pseudomonas fluorescens* SBW25::Gent^{R}. These experiments aim to illustrate how the evolution of AMR can be driven by factors other than the use of antibiotics, reinforcing the importance of concepts such as OneHealth and antibiotic stewardship.

A151

Histidine transport and its role in Cu resistance in *Streptococcus agalactiae*

Ignacio Terán ORCID iD, Matthew Sullivan ORCID iD

School of Biological Sciences, University of East Anglia, Norwich, United Kingdom

Abstract

Streptococcus agalactiae (GBS) is a common commensal microorganism that asymptomatically colonizes the human gastrointestinal and genitourinary tracts in ~30% of healthy adults. It is also a versatile opportunistic pathogen that causes severe disease in pregnant women, neonates, the elderly, and the immunocompromised. To survive within the host, GBS must adapt to both nutrient variations and toxic agents. Copper (Cu), for example, is essential for various biological processes but becomes toxic beyond a certain concentration, causing cell death. GBS can detect increased Cu concentrations within macrophage phagosomes and subsequently activate the expression of various resistance determinants to counteract its toxicity. Mutants in these determinants display reduced virulence in a murine model of disseminated infection, emphasizing the need for precise Cu concentration control. In GBS, this regulation is primarily achieved through the expression of metal transporters and chaperones. However, recent studies have indicated that transport-independent factors also play a role in metal resistance in other pathogenic bacteria. In GBS, it has been suggested that histidine (His) transport via the ABC-type transporter encoded by *hisMJP* also contributes to Cu resistance. Our observations show that a Δ*hisMJP* strain exhibits severely attenuated growth in the presence of Cu concentrations that are subinhibitory for the wild-type (WT) strain, even more so than a mutant strain in *copA*, which encodes a P-type Cu ATPase efflux system critical for GBS virulence. Additionally, high His concentrations can rescue both WT and mutant GBS strains from Cu toxicity, an effect specific to this amino acid.

Session : Knocking Out AMR Forum

A154

Evaluation of antibiofilm activities of the bacteriocin mediated nano-conjugate

Bandita Dutta ORCID iD, Dr. RinaRani Ray ORCID iD

Maulana Abul Kalam Azad University of Technology, West Bengal, Nadia, India

Abstract

Bacterial biofilm is a complex association of bacterial cells give rise to antibiotic resistance. The biofilm matrix preventing the accession of antibiotics, give rise to antibiotic resistance, which becomes a global disaster. Hence, an alternative strategy needs to be adopted to target the biofilm matrix and manage the recalcitrant biofilm forming bacterial pathogens. Bacteriocins, being considered as one of such therapeutic option, are the ribosome mediated proteinaceous toxins having the potential to inhibit the growth of a small range of bacteria and often found to exhibit antifungal, and antiviral properties. Since, the bacteriocins are limited by their narrow antimicrobial range, attempts were made to increase its bactericidal efficacy. In the present study, to address these constraints, bacteriocin was isolated and purified from a new LAB strain which was used for the biogenic synthesis of bacteriocin-capped nano-silver particles. The synthesized nanoparticles were characterizdsed. Furthermore, the efficacies of the AgNP against two nosocomial disease-causing organisms, namely Staphylococcus *aureus* ATCC 23235 and *Pseudomonas aeruginosa* ATCC 10145 were evaluated. It was observed that this biogenic nanoparticle (Bac-AgNP) was able to reduce bacterial biofilm 81.82667±0.03163% (*S. aureus*) and 78.43±0.03796% (*P. aeruginosa*) of the biofilm at concentration 17X10-1µg mL-1. It is able to disrupt the EPS matrix of the bacterial biofilm which was further evaluated via SEM and FTIR analysis. The nano silver bacteriocin was found to be more effective to remove these recalcitrant pathogens.

A155

Evaluating colistin use in Veterinary Medicine

najla alsabali

SFDA, Riyadh, Saudi Arabia

Abstract

Introduction

colistin plays a pivotal role in human and veterinary medicine. It is considered as the last line treatment for infections caused by gram negative bacteria. However, there is an increasing rate of colistin resistance worldwide. Thus, several regulatory authorities set legislation to tackle the problem of colistin resistance. It is proven that resistance transmit from animals to human via consuming food products from animals. It is crucial to restrict the use of colistin in veterinary medicines to prevent the spread of colistin resistance in humans.

Methods

literature review was conducted by reviewing the regulations and restrictions of colistin use in food producing animals in several regulatory authorities around the world. additionally, the percentage of colistin resistance in animals and humans in several countries were reviewed. it is well known that antimcirobial resistance transfer from animals to humans via consuming food produced from animals. thus, it was important to apply restrictions on highest priority antibiotics such as colistin.

Conclusion

Colistin plays a pivotal role in human and veterinary medicine. It is of high importance to set rules and regulations to prevent the misuse of highly important antibiotics such as colistin. It is proven that colistin resistance can transfer from animal to human via consuming food from food producing animals such as meat, egg and milk. Thus, restrictions on using of colistin in veterinary medicine is crucial.

A156

Escape from an antimicrobial CRISPR-Cas9 treatment is dependent on the genetic context of the target gene.

Clàudia Morros-Bernaus[1], Ethan R. Wyrsch[2], Steven P. Djordjevic[2], Anne FC. Leonard[3], David Sünderhauf[1], Stineke van Houte[1]

[1]Environment and Sustainability Institute, University of Exeter, Biosciences, Penryn, United Kingdom. [2]The i3 institute, University of Technology Sydney,, Ultimo, NSW 2007, Australia. [3]European Centre for Environment and Human Health, University of Exeter Medical School, Penryn Campus, Cornwall, United Kingdom

Abstract

Antimicrobial Resistance (AMR) is a global health concern. CTX-M enzymes are a common group of Extended-Spectrum Beta-lactamases (ESBL). The extraintestinal pathogenic *Escherichia coli* sequence type 131 (ST131), responsible for urinary tract and bloodstream infections, is often associated with antibiotic treatment failure. In this context, CRISPR-Cas9 is a promising antimicrobial tool. Here, we explored the use of CRISPR-Cas9 to target a chromosomally carried $bla_{CTX\text{-}M\text{-}15}$ gene in human-associated ST131 isolates.

A CRISPR-Cas9 cassette targeting $bla_{CTX\text{-}M\text{-}15}$ was conjugatively delivered to four *E. coli* ST131 isolates, all of them chromosomally carrying a single $bla_{CTX\text{-}M\text{-}15}$ copy either downstream of the insertion sequence (IS) ISEcp1 or flanked by several equally oriented copies of IS26.

Targeting of $bla_{CTX\text{-}M\text{-}15}$ showed strong antimicrobial activity across isolates when compared to a non-targeting control. However, ST131 escapers that survived CRISPR-Cas9 targeting were readily detected. Escapers through dysfunctional CRISPR-Cas9 were found at low frequency across isolates (~10^{-5}). In contrast, escapers with chromosomal rearrangements, leading to $bla_{CTX\text{-}M\text{-}15}$ loss, were observed at different escape frequencies depending on the genetic context of $bla_{CTX\text{-}M\text{-}15}$: ~$10^{-5}$ for ISEcp1 and between 10^{-4} to 10^{-3} for IS26.

Our work shows that the presence of IS elements associated with the target AMR gene can drive the response to a CRISPR-Cas9 AMR targeting tool. This study contributes to understanding the consequences of CRISPR-Cas9-based AMR targeting in realistic environments and highlights the importance of understanding the genetic context of CRISPR-Cas9 targets to predict outcomes when utilizing these tools.

A157

Use of a visual simulation tool: Veterinary Infection and Prevention through Visualisation (VIPVis), to improve antimicrobial stewardship in the veterinary practice environment.

Rebecca Thomas[1], Kayleigh J. Wyles[2], Alastair Macdonald[3], Dynatra W.D. Subasinghe[1], Mark Chambers[4,5], Roberto La Ragione[1,4]

[1]School of Veterinary Medicine, University of Surrey, Guildford, United Kingdom. [2]School of Psychology, University of Plymouth, Plymouth, United Kingdom. [3]School of Design, Glasgow School of Art, Glasgow, United Kingdom. [4]School of Biosciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom. [5]Department of Bacteriology, Animal and Plant Health Agency, Addlestone, United Kingdom

Abstract

Antimicrobial resistance remains a critical issue for human and animal health, thus novel intervention strategies are urgently sought. Veterinary Infection and Prevention through Visualisation (VIPVis) is a training tool which visually simulates the real-life interactions between humans, animals and pathogens within the veterinary environment by making 'the invisible, visible', in this case, bacterial contamination. A pilot study was performed in an animal hospital to assess whether using the VIPVis app could contribute to lowering bacterial burden throughout the veterinary practice. To measure bacterial burden, environmental swabs (n = 28) were collected from different areas of a variety of rooms throughout the veterinary practice at two time points, pre-intervention, and post-intervention. Swabs were plated onto six types of solid growth media to detect the presence of five clinically relevant veterinary bacterial species and evaluate the overall level of contamination throughout the practice. Detailed analysis revealed that two rooms; the pharmacy and preparation room, had significant ($P<0.05$) reductions in bacterial numbers (CFU/mL) following the intervention. Specifically, significant reductions ($P<0.05$) in bacterial numbers were observed for the pharmacy room and preparation room when swabs were plated onto mannitol salt agar and sheep blood agar, and nutrient agar and sheep blood agar, respectively. Additionally, a trend showing reduced CFU was seen for individual samples across all rooms, despite not reaching significance ($P<0.05$) for each room. The pilot study has shown potential for the VIPVis app to contribute to reductions in environmental bacterial contamination in veterinary practice.

A158

A systematic review to assess the association between the Social Determinants of Health and Antimicrobial Resistance in Europe

Tasleem Damani, Nadya Belenky

University of Greenwich, London, United Kingdom

Abstract

Background

Antimicrobial resistance (AMR) is a global public health threat and a high priority for the World Health Organization to preserve treatment options against infectious diseases. In Europe, the estimated number of resistant infections is growing, resulting in increasing mortality rates and financial burden. Social determinants of health (SDoH) can impact health outcomes by creating inequities within societies and the association between SDoH and AMR needs to be explored to develop strategies towards more equitable healthcare and reduced AMR in vulnerable populations.

Objectives

This systematic review assesses the association between various SDoH and AMR in Europe.

Methods

Key factors associated with SDoH and AMR were searched using EBSCOhost health sciences database. Articles were reviewed using the Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) protocol. Reviewed articles were analysed for themes across articles.

Results

20 articles from 12 European countries met the inclusion criteria. Themes generated included an increase in publications within the field post COVID 19 pandemic. Populations that were disproportionately impacted by AMR included: women, the elderly, individuals from ethnic migrant backgrounds as well as individuals belonging to lower socioeconomic backgrounds. Differing cultural views and perspectives on antibiotic use between healthcare professionals and the public were also associated with AMR.

Conclusion

Associations were found between several SDoH and AMR in Europe. These findings may support the development of interventions targeting the reduction of AMR.

A159

Antibacterial Properties of Gingerol: A Systematic Review and Meta-Analysis on its Role in Ancient and Modern Medicine

Miryam Khazraj, Blessing Anonye

Department of Life Sciences, Manchester Metropolitan University, Manchester, United Kingdom

Abstract

In healthcare, a major issue is the rising number of cases related to antibiotic-resistant illnesses which has significantly increased the demand for natural remedies as alternative treatments. Ginger, also known as *Zingiber officinale*, is known for its therapeutic effects and has drawn significant interest for its revolutionary antimicrobial properties, as well as its value in traditional medicine systems like Ayurveda and Traditional Chinese Medicine. Gingerol, the most active phenolic compound has been shown to possess antimicrobial activity against various pathogens, including Gram-positive and -negative bacteria, demonstrating the potential in combatting drug-resistant infections. This research aims to identify whether gingerol can be a potential alternative treatment for bacterial infections and elucidate possible mechanisms of action.

This research was performed following the guidelines by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. Searches for primary research articles assessing the antimicrobial effects of gingerol against bacteria were performed using PubMed, Web of Science and Scopus databases. The inclusion criteria include research articles that present the antibacterial activity of gingerol using disc diffusion tests and minimum inhibitory concentration determination. Articles not focused solely on gingerol or combinations with other compounds and non-bacterial findings were excluded. Preliminary analysis of studies indicates that gingerol has antibacterial activity and the mechanism of action may be linked to damaging bacterial membranes and cell walls causing the bacteria to lose structural and vital metabolic functions. These results may contribute to the advancement of using plant-based antimicrobial alternatives, thereby addressing the ongoing issue of antimicrobial resistance.

A160

FraB deglycase a novel target for combating multidrug resistant Salmonella enterica serovar typhimurium

Khem Raj Meena ORCID iD

Central University of Rajasthan, Kishangarh, India

Abstract

Salmonella enterica serovar typhimurium is an opportunistic pathogen that causes mild and severe diseases like salmonellosis. *Salmonella enterica* is the most severe and the leading cause of hospitalization and death from foodborne diseases. Diarrhea (caused by *Salmonella* sp.) is also one of the top four causes of morbidity and mortality, especially in children in developing countries. This pathogen (*Salmonella enterica*) is on high priority list of WHO list released in 2024. FraB deglycase of *Salmonella enterica* is a key enzyme in the Fructose asparagine pathway, especially in *Salmonella*. The FraB gene product is encoded in an operon of *Salmonella enterica* responsible for the uptake and utilization of fructose-asparagine (F-Asn), an Amadori product found especially in several human foods. Mutations or Inhibition in fraB deglycase gene cause an accumulation of the FraB substrate, 6-phosphofructose-aspartate (6-P-F-Asp), which is toxic to *Salmonella enterica* serovar typhimurium leading to killing or inhibition of growth. This F-Asn catabolic pathway is found only in the nontyphoidal Salmonella serovars and the fructose asparagine pathway is absent in humans. Thus, targeting FraB with novel lipopeptide-like antimicrobials is expected to be Salmonella-specific, leaving the normal microbiota intact and having no effect on the host. In our study, we have cloned and expressed this FraB gene successfully. In our study, lipopeptide antibiotic molecules also found effective in controlling Salmonella serovar typhimurium. Our study focused on overall antimicrobial resistance in *Salmonella enterica* and possible solution strategies to combat this multidrug resistance *Salmonella enterica* bacterium.

Keywords: Antibiotics, *Salmonella enterica*, MDR, FraB deglycase, Lipopeptide

A161

Capsules, Pumps, and Phages: A New Strategy Against Antibiotic-Resistant *Acinetobacter baumannii*

Anita Tarasenko ORCID iD[1], Bhavya Papudeshi[1], George Bouras[2], Brynley Davies[1], Maoge Zang[1], Abbey Hutton[1], Sarah Giles[1], Susanna Grigson[1], Vijini Mallawaarachchi[1], Bart Eijkelkamp[1], Robert Edwards[1]

[1]Flinders University, Adelaide, Australia. [2]University of Adelaide, Adelaide, Australia

Abstract

Antibiotic resistance poses a global threat, especially with multidrug-resistant (MDR) pathogens like *Acinetobacter baumannii*, which resist treatment through mechanisms like efflux pumps and capsule-mediated defence. Phage therapy, using bacteriophages to target bacteria, offers an alternative for treating these infections. This study identified five novel bacteriophages from sewage and assessed their ability to target A. baumannii efflux pump mutants. Phages were tested on wild-type strains, efflux pump mutants, outer membrane protein FadL mutants, and a capsule mutant to evaluate specificity and therapeutic potential. Genomic analysis confirmed the absence of virulence factors and resistance genes, supporting their clinical suitability.

Transmission Electron Microscopy (TEM) revealed structural diversity: four phages had Siphoviridae morphology, and one had Myoviridae morphology. Notably, efflux pump mutants exhibited thicker capsules, enhancing resistance to phage attack, and indicating a defence adaptation. Host range analysis showed that efflux pump mutations significantly impacted phage infectivity. Capsule density assays further highlighted a correlation between increased capsule thickness and enhanced phage resistance.

Genetic similarity among Siphoviridae phages suggests they could target diverse A. baumannii strains, supporting their clinical application. This study highlights the potential of phages targeting efflux pump mutants as a promising therapeutic strategy against MDR *A. baumannii*. Combining phage therapy with antibiotics could provide an effective approach to managing these challenging infections and advancing our understanding of phage-host interactions in the fight against antibiotic resistance.

A162

Novel beta-lactamases and widespread beta-lactam resistance discovered in untreated wastewater

Alexander Kingdon ORCID iD, Ellie Allman, Anya Breen, Kara D'Arcy, Claudia McKeown, Adam Roberts

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Abstract

Antimicrobial resistance is growing increasingly common in clinical settings, however, many of the resistance genes circulating were already present in the environment. Hence, studying the resistance profiles of environmental microbes could identify novel resistance genes prior to their transfer into pathogenic species. To investigate this further, we screened a set of 884 bacteria isolated from untreated wastewater, for the presence of extended spectrum beta-lactamases, and specifically meropenem resistance. Overall, >50% grew on beta-lactam supplemented agar and ~24% were phenotypically meropenem resistant. We performed follow-up work on eight environmental strains, six Pseudomonas, one Acinetobacter and one Telluria, which all grew on both supplemented agars but did not encode beta-lactamases found in ResFinder or CARD. In contrast, partial matches were found using ARG-ANNOT for half of the strains, while Prokka annotation was used to identify the remaining novel sequences. The six Pseudomonas encoded protein sequences were between 61-77% identical to the closest known AmpC-type beta-lactamase, blaPFL-1. One Pseudomonas strain also contained a sub-class B2 metallo-beta-lactamase, with 88% identity to its closet match blaPFM-1. The Acinetobacter strain encoded a putative class D beta-lactamase with 71% identity to blaOXA666, while the Telluria strain encoded a putative dual class B3-D beta-lactamase with 67% identity to blaMSI-1. These novel beta-lactamase genes were cloned into *E. coli* and the majority could confer resistance to either ceftazidime or meropenem. These genes represent an environmental reservoir of beta-lactam resistance, which could become clinically relevant in the future.

A163

Utilising culture-based methods to monitor three Irish hospitals for antimicrobial resistant pathogens

Stephen Hughes[1], Shannon Carter[1], Michelle O'Reilly[2], Marie Gaffney[2], Cathal Collins[2], John McCarthy[3], Breda O'Brien[3], Nuala O'Connell[3], Aoife Kearney[4], Sinead O'Donnell[4], Fiona Walsh[1]

[1]Maynooth University, Maynooth, Ireland. [2]Cavan Monaghan Hospital, Cavan, Ireland. [3]University Hospital Limerick, Limerick, Ireland. [4]Beaumont Hospital, Dublin, Ireland

Abstract

This project monitors bathrooms in three Irish hospitals as AMR pathogen reservoirs. Sink drains, shower drains and toilets were swabbed, and wastewater was collected from shower drains. Samples were screened for cefotaxime resistant (CTX^R) *Escherichia coli* and *Klebsiella* spp., imipenem resistant (IPM^R) *E. coli* and *Klebsiella* spp., vancomycin resistant *Enterococcus* (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA) using selective agar. AMR pathogens were found in all sites and rooms across all timepoints. 180 CTX^R *E. coli*, 152 IPM^R *E. coli*, 153 CTX^R *Klebsiella* spp., 97 IPM^R *Klebsiella* spp., 25 VRE and 93 MRSA were isolated from shower wastewater. Shower swabs contained 174 CTX^R *E. coli*, 97 IPM^R *E. coli*, 144 CTX^R *Klebsiella* spp., 101 IPM^R *Klebsiella* spp., 52 VRE and 114 MRSA samples. Sinks yielded 180 CTX^R *E. coli*, 120 IPM^R *E. coli*, 156 CTX^R *Klebsiella* spp., 106 IPM^R *Klebsiella* spp., 35 VRE and 95 MRSA. Toilets had 131 CTX^R *E. coli*, 90 IPM^R *E. coli*, 89 CTX^R *Klebsiella* spp., 61 IPM^R *Klebsiella* spp., 27 VRE and 81 MRSA samples. CTX^R *E. coli* was the most abundant pathogen and VRE was least abundant. Shower wastewater yielded most the isolates and toilet swabs yielded least, except in hospital C. Sampling was performed six months after initial sampling for all hospitals and ten months afterwards for hospital A and B which saw reduced isolates except in hospital C. This study concluded that hospital sanitary ware is an AMR pathogen reservoir and measures should be taken to eliminate them.

A164

Proteomic response of *bla$_{CTX-M-15}$* producing *Escherichia coli* in cefotaxime

Chloe McKenna, Monika Subanovic, Dean Frawley, Fiona Walsh

Maynooth University, Maynooth, Ireland

Abstract

Background: The enzyme *bla$_{CTX-M-15}$,* an extended spectrum beta lactamase, has potent hydrolytic activity against the third generation cephalosporin cefotaxime. By using Mass Spectrometry, the proteomic response of a *bla$_{CTX-M-15}$* producing strain of *Escherichia coli* to cefotaxime can be studied, comparing to a susceptible strain of the same species.

Methods: A cefotaxime resistant, *bla$_{CTX-M-15}$* producing *E. coli* and cefotaxime susceptible *E. coli* were grown in x2 their MIC of cefotaxime for 2 hours. The proteins were extracted and digested with trypsin, before being analysed on a Q-Exactive Mass Spectrometer. Raw data files were analysed using software and online tools such as MaxQuant, Perseus and Amica.

Results: Proteins increased in abundance in the resistant *E. coli* included proteins involved in protein synthesis, including 30S subunit proteins such as RpsK, RpsO, RpsE and 50S subunit proteins such as RplA, RplY, RplK. This indicates a reduction of protein synthesis in the susceptible *E.coli.* Proteins increased in the susceptible *E. coli* include shock and stress proteins such as OsmC: osmotic stress protein, YgiW: stress induced protein, CspC, CspA: cold shock proteins, and RbsB, a protein involved in chemotaxis.

Conclusion: Proteins increased in the cefotaxime resistant *E. coli* include ribosome subunit proteins, hence these proteins were decreased in susceptible strain, which indicates a reduction of protein synthesis in the cefotaxime susceptible *E. coli*. Proteins increased in the cefotaxime susceptible *E. coli* included stress and shock proteins, indicating the *E. coli* is trying to survive.

A165

Understanding chromosomal and plasmid interactions of antibiotic resistance genes in *E. coli*

Meghana Srinivas ORCID iD, Fiona Walsh

Department of Biology, Maynooth University, Ireland

Abstract

Plasmid-borne antibiotic resistance genes (ARGs) are the primary mechanism through which bacteria acquire resistance to antibiotics. Under constant selective pressure, these ARGs are thought to integrate into bacterial chromosomes, consequently resulting in the loss of the original plasmid carrying the resistance genes. However, detailed mechanisms driving plasmid-mediated ARG integration, followed by plasmid loss, remain unclear. Recent findings indicate that anti-plasmid systems, involving genes like *apsAB* and *ddmDE*, influence plasmid stability and may play a role in ARG chromosomal integration. These integrations are often associated with the addition of insertion sequences and transposons at multiple sites within the chromosome and plasmid, highlighting potential hotspots for ARG incorporation.

Our study focuses on the integration process of the beta-lactamase genes (*blaCTX-M*) and carbapenemase genes (*bla*OXA) in *Escherichia coli*, a WHO-designated priority pathogen. Using phenotypically well-characterised *E. coli* strains, including one previously unsequenced (DSM 22664), preliminary analyses suggest chromosomal integration of these ARGs, as genes encoding the two ARGs of interest were identified in both chromosomal and plasmid assemblies, along with common insertion sequences and transposon sites identified in both assemblies. Additionally, we aim to identify other genes involved in anti-plasmid systems, many of which remain uncharacterised and lack reference sequences in public databases.

Insights from this research hold the potential to advance strategies for identifying and targeting anti-plasmid systems in *E. coli*, paving the way for alternative approaches to combat antimicrobial resistance.

A166

Resistant and Biofilm Forming *Escherichia coli* from Flooded and Non-Flooded Agricultural Soils

Shauna O'Shea ORCID iD[1,2], Eva Kilcoyne[2], Fiona Walsh ORCID iD[1,2]

[1]Kathleen Lonsdale Institute for Human Health Research, Maynooth, Ireland. [2]Maynooth University, Maynooth, Ireland

Abstract

Little is known about the impact of climate change on antimicrobial resistance. It's predicted that Ireland will experience increased annual flooding due to heavy rainfall events, impacting agricultural lands, increasing the risk of infection due to *Escherichia coli* (*E.coli*).

Agricultural sites (n = 8) were identified and soil samples were taken from flooded and non-flooded areas. Through enrichment, *E. coli* were selected using EMB agar. 16S rRNA PCR was used to determine *E.coli* identity. Disk diffusion and minimum inhibitory concentration methods were used to test antibiotic susceptibility. To test biofilm formation, 96-well plates and crystal violet staining have been utilised. Isolates with antibiotic resistance underwent whole genome sequencing (Novogene).

More *E.coli* (n=47) were isolated from flooded than non-flooded soil (n=15). Almost all *E.coli* (n=14) from non-flooded soils were susceptible to all antibiotics investigated. In flooded soils, 11 isolates exhibited multi-drug resistance, which is plasmid mediated. In total 91% of *E.coli* isolated can form biofilm of varying degree.

A greater number of *E.coli* were isolated from flooded than non-flooded soils, 47 and 15 respectively, suggesting flooded soils are potential reservoirs for *E.coli*. *Escherichia coli* from flooded soils displayed greater antibiotic resistance. *Escherichia coli* from both soil types have the ability to form biofilms.

A167

Antibiotic adjuvants to target chronic P. aeruginosa infections in the cystic fibrosis lung

Charlotte Wragg[1,2], Yiwei Wang[1], Daniel Neill[2], David Hong[1], Jo Fothergill[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]University of Dundee, Dundee, United Kingdom

Abstract

Antibiotic resistance is a global health crisis, resulting in millions of deaths annually and driving the urgent need for new therapeutic strategies. People with cystic fibrosis are especially vulnerable to chronic respiratory infections, with *Pseudomonas aeruginosa* as the predominant pathogen. These infections frequently exhibit resistance or tolerance to empirical antibiotic treatments. This project aims to design and develop antibiotic potentiators to boost the efficacy of existing antibiotics against chronic *P. aeruginosa* infections in cystic fibrosis lungs. By developing these potentiators, we seek to enhance treatment outcomes and address the growing challenge of antibiotic resistance. Through *in silico* and *in vitro* screening, we have identified a primary compound with significant potentiation activity when combined with clinically relevant antibiotics. Clinical *P. aeruginosa* isolates from people with cystic fibrosis serve as the target strains for our investigation. Structural analogues of the primary compound are being ranked based on their potentiating efficacy.

Our *in vitro* studies have demonstrated that these analogues exhibit potentiating activity at moderately low concentrations with low cytotoxicity. However, the mode of action remains unclear. The compounds appear to function as anti-virulence agents, reducing the production of redox-active phenazines and directly reducing biofilm formation by up to 50%

Current results emphasize the potential of these compounds as effective resistance breakers against clinical *P. aeruginosa* isolates. Our ongoing efforts include further investigations into their mode of action, including thermal proteome profiling and extended phenotypic analyses.

A168

Antimicrobial properties of antibiotic conjugated gold nanostars

Jack Homer, Chloe James, M. Alejandra Diaz De Rienzo, Zeljka Krpetic

University of Salford, Salford, United Kingdom

Abstract

The global threat of antimicrobial resistance is one of the most significant public health challenges facing the world today. As pathogenic bacteria develop increasing resistance to current antibiotics, novel therapeutics need to be explored. Metallic nanoparticles are a good candidate to be used as future antimicrobials. Metal nanoparticles in particular offer a new way of approaching antimicrobial therapeutics development, due to the high size and shape tuneability which impacts on the therapeutic potential due to differences in chemical microenvironments, as well as being able to be functionalised with ligands and antibiotics to improve their antimicrobial properties. This research demonstrates the approaches to the rational design of functional gold nanostars and their antimicrobial assessment strategies.

We outline a strategy for assessment of the antimicrobial activity of the antibiotic-conjugated gold nanostars, compared to spherical counterparts used as control, against the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Robust nanoparticle characterisation strategies of the particle-drug interface are also presented (e.g. use of Differential Centrifugal Sedimentation (DCS), Dynamic Light Scattering (DLS), UV-vis spectroscopy, HPLC for drug quantification and electron microscopy (TEM) for particle morphology), alongside studies to determine the antimicrobial properties of different sizes of nanostars with antibiotic combinations. When tested, it was found, in the case of *S. aureus*, that size of gold nanostars effects their antimicrobial properties, with larger gold nanostars exhibiting a lower minimum inhibitory concentration (MIC).

A169

Designing Next-Generation Antimicrobial Nanoparticles: A Novel Approach to Combat Antibiotic Resistance

Heba Elgamodi, Chloe James, Alejandra Diaz de Rienzo, Zeljka Krpetic

School of Science Engineering and Environment, University of Salford, Manchester, United Kingdom

Abstract

The World Health Organisation has identified antimicrobial resistance (AMR) as one of the most significant global health threats to humanity. AMR was responsible for 1.27 million fatalities globally in 2019. Deaths of 10 million every year by 2050 are estimated unless a global response is launched. Nanomaterials have gained significant attention as a viable therapeutic approach to combat resistant microbes. Having unique and tuneable physio-chemical properties, such as size, shape, and surface chemistry, nanoparticles can be adapted to impact a number of biological responses, e.g. intracellular fate, biological membrane interactions, and biodistribution. Coupled with functional ligands and therapeutic molecules e.g. antibiotics, there is a potential of generating powerful next generation therapeutic tools, with strengthened synergistic antimicrobial effects.

This interdisciplinary research focuses on the rational design and development of advanced therapeutic nanoparticles and novel protocols for assessment of copper-based nanoparticles, with unique surface functionalities designed to target and kill pathogens that are at the top priority of the WHO list. The research presented aims to advance the knowledge and understanding of challenges and rational design strategies linked with the application of copper-based functional nanomaterials in combating antimicrobial resistance. Herein, we demonstrate the antimicrobial activity of copper-based nanoparticles designed against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Broth microdilution assays showed that the copper-based nanoparticles conjugated with Vancomycin and Aztreonam had a higher antimicrobial effect against reference strains of *S. aureus* and *P. aeruginosa,* respectively, in comparison to free Vancomycin and Aztreonam.

A170

Investigating the Implication of NAD(P)H Quinone Oxidoreductases in Antibiotic Resistance in *Pseudomonas aeruginosa*

Christina Stylianou, Ali Ryan

Northumbria Univesrity, Newcastle upon Tyne, United Kingdom

Abstract

Antimicrobial resistance (AMR) is one of the most profound global health challenges of the 21st century, threatening public health and the efficacy of modern medicine. AMR occurs when microorganisms, including bacteria, fungi, and parasites, develop resistance to previously effective antibiotics. This phenomenon is especially concerning among Gram-negative bacteria like *Pseudomonas aeruginosa*, a major cause of hospital-acquired infections, particularly in immunocompromised patients. *P. aeruginosa* is known for its resistance to antibiotics, such as aminoglycosides, fluoroquinolones and β-lactams.

NAD(P)H quinone oxidoreductases (NQOs) are enzymes that reduce quinones and other substrates, helping cells manage oxidative stress. Recent studies suggest NQOs play a role in antibiotic resistance by protecting bacteria from oxidative stress-inducing antibiotics like fluoroquinolones and aminoglycosides. This study investigates the role of NQOs in *P. aeruginosa*'s resistance mechanisms using a suicide plasmid system to generate NQO gene knockouts. Complementation of these knockouts will be achieved via Gateway cloning, restoring NQO activity.

The NQO knockouts will be assessed for bacterial growth and antibiotic resistance using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests for levofloxacin, ciprofloxacin, gentamicin, and tetracycline. Additionally, phenotypic assays will evaluate biofilm formation, swimming, swarming, twitching, and pyocyanin production. These experiments will determine whether NQOs influence *P. aeruginosa*'s resistance to oxidative stress-inducing antibiotics and key virulence traits. The results are expected to provide evidence for NQOs as therapeutic targets to combat *P. aeruginosa* infections and mitigate the growing threat of AMR.

A171

Distribution of Antimicrobial Resistance Genes *blaTEM, blaCTX-M, blaVIM,* and *blaCMY* in Irish Farm Soils.

Damien Tansey ORCID iD

Maynooth University, Maynooth, Ireland

Abstract

Background

The β-Lactam class of antimicrobials are used to treat bacterial infections in human and veterinary medicine. As with other classes of antimicrobials, resistance to β-lactams has been reported. Development of plasmid mediated resistance is of particular concern as it allows for the horizontal spread of β-lactam resistance genes, such as *blaTEM, blaCTX-M, blaVIM,* and *blaCMY,* between bacteria.

Soils support a complex ecosystem of micro-organisms. Human activity, such as farming or improper/overuse of antimicrobials, contaminates soils with antimicrobials and forces an evolutionary shift in bacteria towards developing or selecting for antimicrobial resistance.

Methods

Extracted DNA from soil samples were obtained from Irish farms. qPCR was utilised to detect the presence of the *blaTEM, blaCTX-M, blaVIM, a*nd *blaCMY* antimicrobial resistance genes. The data obtained was then used to create a heat map of antimicrobial resistance gene distribution in Irish farm soils across Ireland.

Results/Discussion

The study highlighted the frequency and distribution of several β-lactam class of antimicrobial resistance genes in Irish farm soils.

Conclusion

The data compiled can be used to inform farmers and policy makers of the distribution of antimicrobial resistance genes across Irish farms. The creation of the heat map can be used as a useful visual aid when interacting with the public, farmers and veterinarians.

A172

Mitigation of microplastic-associated antimicrobial-resistant genes and pathogens by chlorination

Yeongmi Park ORCID iD, Hongmok Jo, Si-Kyung Cho ORCID iD

Dongguk University, Goyang, Korea, Republic of

Abstract

The existence of MPs has been extensively identified in almost every aquatic environment with various magnitudes. Furthermore, the ubiquity of microplastics (MPs) in aquatic environments has raised significant concerns regarding their roles as vectors for antibiotic-resistance genes (ARGs) and antibiotic-resistant pathogens (ARPs). This study investigated the mitigation of ARGs and ARPs associated with field-collected MPs through chlorination using free available chlorine (FAC) at varying concentrations. FAC effectively reduced the absolute abundance of ARGs on MPs by up to 99.69%, although the relative abundance of certain ARGs persisted or increased after treatments. Results revealed that the three-dimensional structure of biofilms on MPs significantly influenced FAC efficacy, with interior biofilm bacteria demonstrating greater resistance than outer biofilm. Additionally, FAC induced fragmentation of MPs, particularly increasing the proportion of particles smaller than 100 µm. Notably, ARGs such as *sul*1 and *erm*B showed substantial reductions in absolute abundance, whereas *erm*C and *sul*2 exhibited less reduction, highlighting the complexity of disinfection in MP-associated biofilms. Results underscore the necessity for further investigation into optimizing disinfection strategies to address complex interactions between MPs, biofilms, and ARGs while minimizing ecological risks associated with MPs in wastewater effluents. These findings underscore the need for optimizing disinfection strategies to mitigate ARG dissemination and address environmental risks posed by MPs in wastewater effluents.

A173

Antimicrobial resistance of phytobacteria isolated from local market spinach deploying a culturomics approach

Stacey Duvenage ORCID iD, Dilsha Dineshkumar, Hayley Thompson ORCID iD

Natural Resources Institute, University of Greenwich, Chatham, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a global issue that poses a serious threat to public health. Studies mainly focus on AMR of human bacterial pathogens, but there are limited studies investigating AMR of phytobacteria. The aim of this study was to isolate phytobacteria from spinach using a culturomics approach. Novel vegan media along with growth conditions, such as temperature, time, and atmospheric conditions, was used in conjunction with high-throughput MALDI-TOF, to enhance the isolation and identification of phytobacteria. Like previous findings, Pseudomonadaceae, Enterobacteriaceae, and Bacillaceae were the most abundant bacterial families identified. The diversity of bacteria isolated from the three vendors differed, with Vendor C exhibiting more diversity than Vendor A.

Following AMR testing, using antibiotic susceptibility disc diffusion and comparison to published breakpoints, 36.4% of Pseudomonadaceae (n=22) and 75% of Enterobacteriaceae (n=16) were multidrug resistant (MDR, resistant to three or more classes). Six Pseudomonadaceae, including *Pseudomonas fluorescens* (n=2), *Pseudomonas grimontii* (n=1), *Pseudomonas mandelii* (n=1), *Pseudomonas putida* (n=1) and *Pseudomonas synxantha* (n=1) were resistant to four out of eight antibiotics. These six Pseudomonadaceae were all resistant to monobactams, co-amoxiclav and cefepime, a fourth-generation cephalosporin. Among the MDR resistant Enterobacteriaceae, *Enterobacter bugadensis* (Vendor A) and *Rahnella woolbedingensis* (Vendor C) were resistant to seven out of 12 antibiotics and were the most resistant phytobacteria tested. Both species were resistant to ampicillin, amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, cefoxitin and ceftazidime, a third-generation cephalosporin. Results highlight that environmental, non-clinical bacteria could act as reservoirs of AMR genes, highlighting the need to test natural reservoirs of AMR.

A174

The assessment of P. aeruginosa virulence genes in models reflecting the CF lung

Tegan Hibbert ORCID iD[1], Hollie Leighton[1], Grace Ritchie[1], Daniel Neill ORCID iD[2], Joanne Fothergill ORCID iD[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]University of Dundee, Dundee, United Kingdom

Abstract

Anti-virulence therapeutics represent a novel approach to treating *P. aeruginosa* cystic fibrosis lung infections. However, many of the approaches used for studying traditional antimicrobials may not be applicable to anti-virulence agents. Furthermore, the effectiveness of anti-virulence therapeutics in conditions relevant to the CF lung is poorly understood.

This work assesses the expression of various anti-virulence genes (*lasR, rhlR, lasB, pqsA, exoS, exoT, exoY, pvdL, fpvB)* in *P. aeruginosa* PAO1, PA14 and LESB58 grown in a variety of models including as a monoculture in Synthetic CF Media 2 (SCFM2) and CF lung media (CFLM) across several time points (2h, 6h, 24h, 72h), a polymicrobial aggregate biofilm model (24h) with *S. aureus* and *C. albicans* and a polymicrobial *ex vivo* pig lung (EVPL) model (24h) at varying oxygen concentrations. The spatial structure of the polymicrobial EVPL is also assessed using cryo-SEM.

Our results indicate that *P. aeruginosa* virulence gene expression is influenced by external factors, with the expression of *rhlR,* for example, being upregulated in LESB58 grown in the polymicrobial biofilm model compared to an SCFM2 monoculture model (P <0.0001). Our results also indicate an altered morphology of the polymicrobial EVPL at different oxygen concentrations, with *C. albicans* exhibiting a spore morphology in aerobic conditions and a hyphal morphology in anaerobic conditions.

Our work increases understanding of the dynamics of the *P. aeruginosa* virulome in models reflecting the CF lung and assesses the impact of external influences on virulence gene expression, which will aid in the development of anti-virulence therapeutics.

A175

TB or not TB: Diagnosing Tuberculosis in West Africa to inform immediate drug choice

suzie hingley-wilson ORCID iD[1], Augustina Sylverken[2], Wellington Owusu[1], Jessie Carpenter[3], Ruben Riano[3], David Rimer[3], Arnoud van Vliet[1], Natalie Riddell[1], Aurore Poirer[1], Roberto LaRagione[1], Graham Stewart[1]

[1]University of Surrey, Guildford, United Kingdom. [2]KNUST, Kwame Nkrumah, Ghana. [3]VIDIIA, Guildford, United Kingdom

Abstract

Often called the forgotten pandemic, Tuberculosis (TB) is a huge global issue with over 1.3 million deaths every year, of which over 80% are due to fully drug-sensitive TB causative agents (W.H.O. global health report, 2023). Much of the global burden of TB is in West Africa where there is an unusual mix of TB-causing lineages from the *Mycobacterium tuberculosis* complex (MTBC), specifically *M. tuberculosis (Mtb), M. africanum (Maf)* and *M. bovis (Mb).* Current diagnostic assays in West Africa do not routinely differentiate these lineages or mixed infections, which is a requisite for correct treatment administration. We analysed over 7000 genomes to identify lineage-specific genes and developed a rapid low-cost, portable, species-resolution diagnostic test for TB to inform drug selection. Testing on sputum samples from 341 clinically confirmed active TB patients revealed rates of 24.9% Mtb, 23.4% Maf, 1.8% Mb and a surprisingly high number of mixed lineage-infections at 5.9%. The Loop-mediated isothermal amplification (LAMP) -based assay's Limit of Detection (LOD) was highly sensitive across lineages (1.37-3.14 copies/µl). In addition, a rapid DNA extraction device (< 1 minute) showed promising results on sputum samples. This multiplex assay will allow speciation of MTBC lineages in low-resource regions, providing rapid differentiation of TB infections to select appropriate medication at the earliest possible time point. It will also be useful in epidemiological surveillance studies providing reliable information on the prevalence of TB lineages as well as identifying difficult to treat cases of mixed-lineage tuberculosis infections.

A176

Mining Environmental Samples for Anti-Stenotrophomonas maltophilia Bacteriophages of Therapeutic Use.

Ayesha Bibi, Grace Plahe, Chloe E James, Andrew Martin, Richard Birtles

School of Science, Engineering and Environment, University of Salford, Salford, United Kingdom

Abstract

Stenotrophomonas maltophilia, an intrinsically multidrug-resistant (MDR) bacterium, is commonly found in natural environments but has emerged as a significant opportunistic pathogen. Infections are increasingly reported in healthcare and community settings, particularly in the respiratory tracts of cystic fibrosis (CF) patients, where MDR characteristics complicate treatment. Advances in bacteriophage therapy offer promising alternatives to antibiotics for managing CF pathogens such as Pseudomonas aeruginosa and Non-Tuberculosis Mycobacteria (NTM). Anti-Stenotrophomonas phages have been identified, and prophages within S. maltophilia genomes show potential for novel therapeutic applications.

This study explores the diversity of antimicrobial resistance and prophage carriage in S. maltophilia isolates from environmental sources across Greater Manchester. Initial findings reveal that S. maltophilia is commonly found in local soil and water. Of 33 soil samples collected from Whitworth Park, Manchester, 54.5% (18 samples) yielded positive cultures that were confirmed as Stenotrophomonas through Sanger sequencing. Ongoing sample collection will further assess regional diversity.

Future work will focus on identifying, inducing, and isolating active bacteriophages from S. maltophilia isolates and environmental samples. These phages will be evaluated for their therapeutic potential against clinical S. maltophilia isolates from chronic respiratory infections.

A177

An evaluation of screening methods for the detection of ESBL-producing *E. coli* and *K. pneumoniae* in environmental samples from healthcare settings

Esther Picton-Barlow ORCID iD[1], Claudia McKeown[1], Sally Forrest[1,2], Sarah Gallichan[1], Maria Moore[1], Joseph M Lewis[1], Fabrice E Graf[1], Nicholas A Feasey[1,2]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom. [2]University of St Andrews, St Andrews, United Kingdom

Abstract

Extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E) are multidrug-resistant bacteria responsible for an increasing number of infections, particularly in healthcare settings. The acquisition routes for these bacteria are not well defined, underscoring the need for a One Health surveillance approach, including environmental surveillance. Effective surveillance relies on laboratory methods that are both sensitive and specific to accurately detect ESBL-E from complex microbial landscapes.

To support our TRACS-Liverpool study, aiming to understand the transmission dynamics of ESBL-E in healthcare settings across Liverpool, we have evaluated methods for screening environmental samples for ESBL-E. Our samples were collected from shower heads, shower drains, sinks, toilets and high-touch surfaces in hospitals and care homes. We assessed pre-enrichment and selective plating steps by testing two pre-enrichment durations (4 vs 18 hours) and broths (Tryptic Soy, Buffered Peptone Water), as well as four selective agars (MLGA, CHROMagar, MacConkey, SCAI). MALDI-TOF MS was used to confirm species identification.

Our results show that extended pre-enrichment enhances screening sensitivity, enabling the detection of ESBL-E from a higher proportion of samples. In addition, preliminary data indicate that MLGA supplemented with cefotaxime offers the highest combined sensitivity and specificity.

The complexity of microbial communities in environmental samples challenges screening methods. Choosing an agar most suitable for this sample type reduced the number of samples requiring more costly and time-consuming identification confirmation. Screening challenges also arise from low abundance bacteria in environmental samples, but extended pre-enrichment increased bacterial load to improve detection accuracy.

A178

Modulating the Inflammatory Response in Respiratory Tissue: Manuka Honey as a Treatment for Chronic *Pseudomonas aeruginosa* respiratory Infections

Michelle Hardman[1], Philip Butterick[1], Angharad Green[2], Daniel Neill[3], Thomas Wilkinson[1], Rowena Jenkins[1]

[1]Swansea University, Swansea, United Kingdom. [2]University College London, London, United Kingdom. [3]University Of Dundee, Dundee, United Kingdom

Abstract

Background: Chronic lung infections in people with cystic fibrosis (CF) are caused by the multidrug-resistant pathogen *Pseudomonas aeruginosa*. Given its resistance to conventional treatments, there is need to explore alternative therapies. Manuka honey, known for its antimicrobial properties, shows promise. This study evaluated manuka honey's effects on inflammation in chronic *P. aeruginosa* infection using an *in vivo* murine model.

Methods: A chronic *P. aeruginosa* murine lung infection model was treated with 30% manuka honey, ceftazidime (8mg/mL), and their combination, delivered intranasally alongside uninfected controls. Bacterial load assessed via total viable counts, and inflammatory response measured through markers including VEGF, CXCL/MIP2, and MPO at 24- and 72-hours post-treatment.

Results: In the murine model, combined honey and ceftazidime treatment significantly reduced bacterial load in the nasopharynx and lungs at 24 hours ($p \leq 0.05$) and in the nasopharynx alone at 72 hours when compared to an infection control. MPO levels were significantly lower at 72 hours in the nasopharynx and at 24 hours in the lungs ($p \leq 0.05$). CXCL/MIP2 was significantly reduced in the nasopharynx at 24 hours with honey alone ($p \leq 0.05$), and VEGF decreased significantly with combination treatment ($p \leq 0.05$). TNF-α levels increased in both honey-treated groups ($p \leq 0.05$), while IL-6 levels were unaffected.

Conclusion: These findings indicate manuka honey, when combined with ceftazidime, can significantly reduce bacterial burden and inflammation in chronic *P. aeruginosa* lung infections. Highlighting its potential as a therapeutic for managing chronic, antibiotic-resistant lung infections in individuals with persistent lung disease.

A179

Elucidating the novel structure of *P. aeruginosa*'s Pyocin S3, and investigating its role

Jessica Lester ORCID iD, Siyu Wu, Christopher Penfold ORCID iD, Kim Hardie ORCID iD

The University of Nottingham, Nottingham, United Kingdom

Abstract

Bacteriocins are a group of bacterial derived proteins which have potent killing effects on their target bacteria. These proteins are specific to the species that produces them, and only interact with their specified target on particular strains. Colicins, produced by *E. coli*, have been very well studied. The bacteriocins of ESKAPE pathogen *Pseudomonas aeruginosa* have yet to be fully characterized. Pyocin S3 is a bacteriocin produced by *P. aeruginosa* that has been implicated in intraspecies competition. Due to its specificity, potency and lethality amongst strains of *P. aeruginosa*, pyocin S3 is an excellent potential target as a novel antimicrobial for the treatment of Cystic Fibrosis associated infections. The structure and specific function of pyocin S3 are still yet to be determined. Here, x-ray crystallography has been utilised to determine the native structure of pyocin S3. Additionally, the function of pyocin S3 has been evaluated in silico and in vitro, using AlphaFold 3 plus functional assays . The crystal structure of the full-length native pyocin S3 and its active domains (the DNase-Immunity complex) have been elucidated to reveal a novel non H-N-H endonuclease active site. The minimal inhibitory concentration of pyocin S3 has been determined in planktonic cultures. The effectiveness of pyocin S3 against *P. aeruginosa* biofilms is under investigation. Our future aim is to determine if pyocin S3 could be used in single therapy applications, or as a part of a larger cocktail of antimicrobials to treat CF *P. aeruginosa* infection.

A180

Process development of a phage therapy product targeting *Klebsiella pneumoniae*

Hannah Beadle, Lee Metcalf

CPI, Darlington, United Kingdom

Abstract

Klebsiella pneumoniae has been identified by the WHO as an ESKAPE pathogen, due to its highly virulent and antibiotic resistant nature, therefore research and development into new treatment areas against this pathogen are vitally important. Phage therapy is a key alternative treatment to antibiotics that has been recognised by the UK government in the Parliamentary report published January 2024 and that is already being used around the world. In the UK, clinical trials are required to test the efficacy of the phage therapy and evidence that they can be used as treatment, which requires the phage to be manufactured to good manufacturing practise (GMP) standard. This has restricted phage therapy in the UK as there is a lack of regulatory guidance for phage production at GMP standard. The aim of this study was to develop a purification process which reduces residual host contaminants to an acceptable level using two chromatography steps, and qPCR as a high throughput analytical method for phage titre determination. These techniques have been used previously in GMP processes and are scalable, which would allow this process to move to GMP more quickly therefore enabling clinical trials to take place sooner. The ultimate goal is to use this process to drive the phage manufacturing industry in the UK, with CPI being ideally placed being already situated in a GMP facility.

A181

SafePhage: Engineering Synthetic Phages with Intrinsic Biocontainment

Christian Harrison[1], Ryan Cochrane[1], Maisie Czernuszka[1], Rosanna Wright[1], Claudia Igler[1], Stefan Hoffmann[2], Patrick Cai[1], Michael Brockhurst[1]

[1]University of Manchester, Manchester, United Kingdom. [2]Wageningen University & Research, Wageningen, Netherlands

Abstract

Phage therapy offers a promising solution to the threat posed by rising rates of antimicrobial resistance. However, synthetic or engineered phages may overcome some of the limitations of natural phages, providing more effective therapeutic options. To safely employ synthetic phage therapy, robust biocontainment strategies are essential to ensure the responsible and controlled use of this potentially transformative technology.

We aim to develop the foundational technologies for different configurations of biocontained phages using top-down gene editing and bottom-up synthetic modular genome assembly techniques. The efficacy of the synthetic phages will be assessed using large-scale screens against a panel of clinical *Pseudomonas aeruginosa* isolates. The robustness of different biocontainment strategies will be quantified using high-throughput evolutionary screening of phage escape mutation. The effects of phage biocontainment and additional antimicrobial genetic cargoes on the dynamics and mechanisms of bacterial resistance evolution to the synthetic phages will also be investigated.

The impact of synthetic phage therapy is likely to span multiple sectors including health, agriculture and biotechnology. We believe the development of biocontainment strategies will be critical to ensuring the safety of synthetic phages to patients, the public and the wider environment.

A182

Making use of antibiotic prescription datasets: predictions, trends and analysis

Anjali Vasudevan, Jan Kreft

University of Birmingham, Edgbaston, Birmingham, United Kingdom

Abstract

Antimicrobial resistance (AMR) has been a cause of concern since 1950. Widespread consumption of a variety of antibiotics leads to the selection of multi-drug resistant bacteria and the slow discovery of new antibiotics and market failure forces us to come up with ways to reduce the spread of antimicrobial resistance. In the UK, the National Health Service (NHS) monitors all prescriptions including antibiotics and this information is publicly available through the NHS Business Service Authority (BSA) website. In this project, we use these prescription data to calculate the total amount of each antibiotic prescribed by each General Practice (GP) in England in each month. Then we combine these totals of antibiotics prescribed with local population data available from the Office for National Statistics (Lower Super Output Areas (LSOAs) consisting of 400-1200 households) to infer the amount of antibiotic prescribed per person. This, combined with the amount of sewage produced per person, can be used to predict the concentrations of antibiotics entering the sewage treatment plants : for example, we predicted that a global concentration of 0.0297mg/L of amoxicillin (the most prescribed antibiotic) enters the WWTPs of England for the month of December 2021) We are currently analysing the antibiotic prescription trends over time and regional differences across England. The next step will be to identify associations of antibiotic usage with potential socio-demographic drivers and with resistance patterns for England.

A183

Global Lineage Dynamics of Antimicrobial Resistance in *Staphylococcus aureus*: Insights from a One Health Study in Uganda

Tim Lee, Bryan Wee, Adrian Muwonge, J. Ross Fitzgerald

Roslin Institute, Edinburgh, United Kingdom

Abstract

Staphylococcus aureus is a globally significant pathogen with a high burden of antimicrobial resistance (AMR), posing serious public health risks. This study uses a One Health framework to explore *S. aureus* diversity and resistance in Uganda, integrating human, animal, and environmental data to understand AMR dynamics. We analyzed isolates from hospitals in Jinja and Mbarara, as well as community and animal populations, using whole-genome sequencing (WGS) and public genome repositories. Comparative genomic analysis and phylogenetic reconstruction were used to assess lineage evolution and global distribution patterns.

Among the 46 sequenced isolates, clonal complexes CC152 and CC121 were predominant, with the CC121 single locus variant (SLV) ST2430 found only in the Uganda isolates. The tet(M) gene was uniquely identified in ST2430 but not in the primary ST121s, and methicillin-resistant *S. aureus* (MRSA) was detected in CC152 SLV ST1633. Global phylogenies of 392 CC152 and 741 CC121 isolates revealed geographic correlations in AMR profiles, linking local antibiotic use to specific resistance patterns, including tetracycline resistance. A host shift was detected in CC152, with human-associated strains found in bovine samples. Further findings indicate multiple independent acquisitions of the dfrG resistance gene across the phylogenies of both CC121 and CC152.

These results demonstrate evolving *S. aureus* epidemiology in Uganda and emphasize the need for integrated AMR surveillance across sectors. A One Health approach is essential for mitigating resistance spread and identifying potential cross-host transmission.

A184

Antimicrobial resistance in *Escherichia coli* isolates from poultry farms in Kaduna, Nigeria

Amelie Delaitre[1], George Siegwart[1], Chioma Achi[1], Kate Cook[1], Edward Portal[1], Teresa Iannetelli[1], Shonnette Premchand-Branker[1], Timothy Beard[1], Mei Li[1], Adeyinka Alagbe[2], Deborah Olayioye[3], Naomi Evina Ewofere[4], Timothy R Walsh[1], Seniyat L Afegbua[3,4], Kirsty Sands[1,5]

[1]Ineos Oxford Institute for Antimicrobial Research, Department of Biology, University of Oxford, Oxford, United Kingdom. [2]Michdave Agroservices, Kaduna, Nigeria. [3]Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria. [4]Department of Biotechnology, Nigerian Defence Academy, Kaduna, Nigeria. [5]Division of Infection and Immunity, Department of Medical Microbiology, Cardiff University, Cardiff, United Kingdom

Abstract

Antimicrobial resistance (AMR) poses a significant threat to public health, and the emergence of resistant bacteria in agriculture is particularly concerning. The inappropriate use of antimicrobials in livestock production drives AMR, where their usage as prophylaxis and growth promoters is employed to limit production and economical losses. By investigating the prevalence and resistance patterns of *Escherichia coli* isolates from poultry farms in Kaduna, we aim to elucidate the extent of AMR and its potential implications on animal and human health.

Environmental samples were collected from poultry farms and *E. coli* strains were isolated from multiple colony picks per plate to capture intraspecies diversity. Antimicrobial susceptibility was assessed through broth dilution methods against 108 antimicrobials commonly used in veterinary medicine. ERIC-PCR was used to investigate the clonal relationship between isolates, while subsequent gDNA extraction and long-read sequencing will allow characterisation of underlying molecular mechanisms of resistance.

214 *E. coli* strains were recovered, where molecular differentiation provided evidence of intraspecies diversity amongst samples. Resistance to several key antibiotics was observed, where enrofloxacin (88.6%), sulfamethoxazole (77.3%), gentamicin (47.5%), doxycycline (27.2%), and cefazolin (25.2%) MICs were 8-fold greater than those of reference strain ATCC 25922, highlighting the emergence of resistant isolates within poultry-production farms, where the highest levels originated from litter. By harbouring resistance, these isolates act as AMR reservoirs, further emphasising the importance of adopting One Health approaches. These results stress the need for surveillance to mitigate emergence of AMR from livestock production, where transmission to humans poses a global health risk.

A185

Genomic insights into antimicrobial resistance in *Pseudomonas aeruginosa* isolates from a global clinical trial

Niamh Harrington[1], Anastasia Kottara[2], Kendall Cagney[1], Matthew Shepherd[3], Taoran Fu[3], Rebecca Hull[4], Dylan Childs[5], James Chalmers[4], Joanne Fothergill[1], Michael Brockhurst[3], Steve Paterson[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Manchester Medical Microbiology Partnership, Manchester, United Kingdom. [3]University of Manchester, Manchester, United Kingdom. [4]University of Dundee, Dundee, United Kingdom. [5]University of Sheffield, Sheffield, United Kingdom

Abstract

Pseudomonas aeruginosa is a major opportunistic pathogen associated with respiratory infections. The rising incidence of antimicrobial resistance (AMR) in *P. aeruginosa* has led to treatment challenges, with limited effective therapeutic options available. We have performed whole genome sequencing of 4,218 *P. aeruginosa* isolates from 180 bronchiectasis patients, enrolled in an unsuccessful international clinical trial for a novel antimicrobial therapy, to investigate preexisting resistance genome features and the mechanisms of AMR evolution *in vivo*. The cohort included patients from countries in Europe, Australia, North America and Asia (including the UK, USA, Israel and Taiwan), representing the largest sequenced collection of bronchiectasis *P. aeruginosa* isolates to date.

Our analysis revealed substantial genetic diversity between patients, with 77% of sequence types unique to individual patients and a low prevalence of epidemic strains (detected in 7% of patients). At baseline, a high prevalence of AMR determinants - including multidrug efflux pump genes and fluoroquinolone resistance mutations - was observed across all regions. During the trial, further resistance mechanisms emerged, primarily driven by *de novo* gain of single nucleotide polymorphisms (SNPs) associated with AMR (detected in 33% of treatment group patients), which resulted in treatment failure.

Our findings show the high incidence of antimicrobial resistance genome features in *P. aeruginosa* across global regions and provide evidence for the mechanisms of AMR evolution occurring in patients. This highlights the importance of antimicrobial stewardship and genomic surveillance to inform clinical trial design for novel antimicrobials, as well as highlighting potential treatment targets to reduce AMR evolution.

A186

Human Health to Animal Health: Interim Results from the Analytical Validation of Rapid Direct from Urine and Urine Isolate Microcapillary Antimicrobial Susceptibility Testing (AST)

Sarah Helen Needs[1,2], Julie Hart[1,2], Hoyin Lam[3], Oliver Hancox[1,2], Stephen Kidd[3], Alexander Edwards[4,2]

[1]University of Reading, Reading, United Kingdom. [2]Astratus Limited, Reading, United Kingdom. [3]Hampshire Hospitals NHS Foundation Trust, Basingstoke, United Kingdom. [4]University of Southampton, Southampton, United Kingdom

Abstract

Background

Standard manual culture AST methods are laborious with slow turnaround (2-7days+). Automated clinical rapid AST products have emerged but remain expensive. Faster, higher throughput, less labour-intensive, accurate and affordable AST is still needed for both human and veterinary clinical medicine as part of a One Health approach. Astratus Limited was spun out of University of Reading (2024) to deliver a novel testing platform to meet this need. We present for the first time, interim findings from our analytical validation.

Methods

Analytical validation was conducted on urine samples and isolates from patients with suspected Urinary Tract Infection (UTI). Samples were spot plated onto Chromoagar UTI brilliance to obtain a total viable count, reference methods were conducted alongside. For diagnostic remnants, disc diffusion (EUCAST v12.0), for fresh samples broth microdilution according to ISO 20776-1:2019 and ISO 20776-2:2019 for results comparison.

Results

Interim analysis showed 96% sensitivity (95% CI: 87-99%) and 99% specificity (95% CI: 97-100%) for detecting microbial growth in urine for samples >10^5CFU/mL (n=367 samples. 129 considered positive >10^5 CFU/mL & 238 negative <10^5 CFU/mL by bacterial plate counts). Moreover, samples with growth of single Enterobacterales showed categorical agreement of ampicillin 90%; amoxycillin 95%; nitrofurantoin 100%; trimethoprim 95%; ciprofloxacin 100%, cefalexin 95% for microcapillary AST direct from urine compared to disc diffusion.

Conclusions

The unique patented properties of our microcapillary technology enables rapid, accurate AST at low cost. The scalable instrument configuration provides high throughput capability, offering an innovative platform to improve productivity, digitise results, and optimise antimicrobial use.

A187

Detection of antibiotic-resistant WHO-priority pathogens in drinking water sources in Scotland

Muriel Chaudhri

Edinburgh Napier University, Edinburgh, United Kingdom

Abstract

AMR is a leading cause of mortality worldwide. The WHO recommends a one-health AMR surveillance approach that includes the environment. As this sector is still understudied, this project aimed to determine the presence of resistant WHO-priority pathogens in surface waters used for drinking water abstraction.

Three rivers, three reservoirs, and one loch located across Scotland were selected based on pollution risks. Total and ESBL-producing *E. coli* were quantified using membrane filtration and incubation on TBX agar (with/without 4mg/L cefotaxime (CTX)). An enrichment approach with antibiotic-amended broths was used to detect meropenem and CTX-resistant *Acinetobacter* spp., *P. aeruginosa*, and coliforms, colistin-resistant coliforms, and vancomycin-resistant *Enterococcus* spp.

E. coli were recovered at six out of seven sites. According to the EU Drinking Water Directive, water qualities ranged from very pristine (<2 CFU/100ml) to moderately contaminated (20-200 CFU/100ml) and one river was contaminated (>200 CFU/100ml). CTX-resistant *E. coli* were only recovered from one source, and at a very low proportion (0.4%).

CTX-resistant *P. aeruginosa* were confirmed in one, CTX-resistant *Klebsiella* spp. in two, colistin-resistant *E. coli* in one, and colistin-resistant *Klebsiella* spp. in three sources. Presumptive vancomycin-resistant *E. faecium/faecalis* were recovered from two sources.

To understand public health implications on a national scale, a point-prevalence study across two hundred sites will follow. All priority pathogens need to be quantified to assess risks accurately. Sources of resistant organisms need to be determined to inform intervention strategies.

A188

Uncovering the antimicrobial and antibiotic potentiating activity of the artificial sweeter Saccharin.

Rubén de Dios[1], Bhupender Singh[2], Christian Lentz[2], Mohamed Soliman[1], Jie Han[1], Ronan R McCarthy[1]

[1]Brunel University of London, London, United Kingdom. [2]The Arctic University of Norway, Tromso, Norway

Abstract

It is widely recognized that there is an urgent need for innovative therapeutic approaches to combat multi-drug resistant bacterial infections. Saccharin, a commonly used artificial sweetener, has shown remarkable potential in inhibiting the growth of a wide range of clinically relevant pathogens. When tested against multi-drug-resistant (MDR) strains, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, saccharin exhibited a dose-dependent inhibitory effect on bacterial growth. Additionally, saccharin was found to inhibit biofilm formation, particularly in *A. baumannii* and *P. aeruginosa*, and even reduced biofilm biomass in established biofilms and polymicrobial biofilms, suggesting its potential use in treating chronic infections associated with biofilm formation.

Saccharin also demonstrated the ability to impair virulence factors, such as twitching motility in *A. baumannii*. It also disrupted bacterial cell morphology and compromised membrane integrity. Furthermore, saccharin enhanced the penetration of fluorescent derivatives of penicillin and vancomycin across the bacterial cell membrane. Critically, saccharin was shown to overwhelm native resistance mechanisms. When carbapenems were co-administered with sub-lethal concentrations of 1.5% saccharin, carbapenem-resistant *A. baumannii* strains exhibited a decrease in antibiotic resistance, with the MICs of doripenem, meropenem, and imipenem falling below the EUCAST breakpoints.

Saccharin-loaded hydrogels proved effective in reducing bacterial loads in both biofilms and ex vivo burn wounds, achieving greater bacterial load reductions than commercial silver-based treatments. This suggests that saccharin, particularly in hydrogel form, could be a promising antimicrobial and biofilm-disrupting agent in clinical settings, especially for wound care and the management of antibiotic-resistant infections.

A189

Carbapenem-resistant and Extended-Spectrum β-lactamase-producing *Escherichia coli* from UK Safari Park Baboons: OneHealth Implications for Captive Primates

Anya Breen, Russell Stothard, Alexandra Juhasz, Ralfh Pulmones, Angus More O'Ferrall, Claudia McKeown, Ellie Allman, Pauline Ambrose, Amber Cutcliffe, Adam Roberts

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Abstract

Antimicrobial resistance (AMR) remains one of the most critical global issues we face in the present day. Addressing this crisis requires a One Health approach, recognising the interconnected roles of humans, animals, and the environment in the propagation of antimicrobial-resistant bacteria. In recent years, bacteria producing resistance genes to our most critical antibiotics have been isolated from livestock and companion animals.

We sought to examine the presence of antimicrobial-resistant bacteria in the gut microbiomes of a colony of baboons at Knowsley safari park, where the animals have close, frequent contact with both their handlers and the public. Between 2021 and 2024, 161 faecal swabs and 19 stomach ulcer swabs were collected from the baboons and screened for both extended spectrum β-lactamase-producing (ESBL) and meropenem-resistant Gram-negative enteric bacteria. In total, 28 ESBL isolates, 91 meropenem-resistant isolates, and 23 isolates displaying both phenotypes were identified.

Of these antimicrobial-resistant isolates, 7 *Escherichia coli* strains were selected for further analysis. These were Illumina whole-genome sequenced, and gene prediction using ARIBA identified three of these as multi-drug resistant ESBLs, while the other four strains were found to be phenotypically meropenem-resistant but lacked acquired carbapenemase genes. Through phylogenetic analyses, these *E. coli* strains were found to be genetically similar to human clinical isolates from hospitals in the United Kingdom. These findings highlight the transmissibility of dangerous antimicrobial-resistant pathogens between humans and captive animals.

A190

Vertical vs horizontal dynamics of bacteria and AMR using Nanopore in the Malawian poultry network

Vesa Qarkaxhija[1], Bryan Wee[1], Williams Mwantoma[2], Harry Milala[3], Ronald Chitatanga[3], Kathryn Kamwana[2], Mike Luwe[3], Chikhulupiliro Chimwaza[3], Stella Mazeri[1], J. Ross Fitzgerald[1], Adrian Muwonge[1], David L. Gally[1]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]Central Veterinary Lab Malawi, Lilongwe, Malawi. [3]Public Health Institute of Malawi, Lilongwe, Malawi

Abstract

Understanding the complex epidemiology of WHO-priority pathogens such as *Escherichia coli* and their antimicrobial resistance (AMR) patterns relies on holistic One Health approaches. Interfaces between bacterial hosts and environments in agricultural networks can result in spread and coalescence of antimicrobial resistance gene (ARGs). Our objective here is to evaluate the contribution of 'vertical' transmission of resistance from founder flocks at the apex, down to multipliers and small-scale farmers, compared to 'horizontal' introduction between humans, animals, and the environment, along the network.

In this study we have sampled a hierarchical national breeding structure across the central region of Malawi. We generated antibiotic enriched long-read metagenomes from 24 poultry samples and combined this with nanopore long-read whole genome sequences (WGS) of 180 *E. coli* from human, poultry and environmental isolates. This unique sample set was compared with publicly available *E. coli* WGS exploring the prevalence of ARGs and strains within the poultry network compared to regional circulating dynamics and clinical cases. The use of long-read sequencing allowed the context of both gut microbiota and isolate ARGs to be determined, with a focus on plasmids and their distribution in the network.

Through a combination of structured sampling, long-read whole genome and metagenomic sequencing, we have characterised AMR flow in *E. coli* and its domicile microbiome from a 60-year-old community poultry breeding system in Malawi.

A191

From estuarine beds to the lab bench: seagrass microbiomes in the quest for new antibiotics

Rutendo Mupfumi, Grant January ORCID iD, Sean Kelly ORCID iD, Leire Dafis-Sagarmendi, Kathryn Burdon, Mathew Upton ORCID iD

University of Plymouth, Plymouth, United Kingdom

Abstract

Antimicrobial resistance has become a global public health concern and new anti-infective agents are needed to overcome this "silent" pandemic[1]. Natural products (NPs) are crucial antimicrobial agents and offer the most promising source for drug discovery compared to synthetic compounds[2]. In this study, seagrass from the River Yealm estuary, Devon, United Kingdom, and their microbial symbionts were investigated for antimicrobial activity.

A bacterial collection produced from local-UK seagrass was screened for antimicrobial activity using phenotypic assays against ESKAPE pathogens. Isolates with promising bioactivities were triaged for further characterisation including "One Strain Many Compounds (OSMAC)" fermentations, *in vitro* bioassays, metabolomics, whole genome sequencing, and *in silico* mining for biosynthetic gene clusters (BGCs).

One promising isolate, *Paenibacillus peoriae*, or SG1, displayed preliminary antibacterial and antibiofilm activities against Gram-positive and -negative pathogens. OSMAC experiments were conducted, and crude extracts fractionated using reverse-phase chromatography, with active fractions currently undergoing bioactivity screening. Untargeted metabolomics and molecular networking by HR-LC-MS/MS will be used to chemically dereplicate the compounds of interest. The genome was sequenced and antiSMASH prediction revealed BGCs, which could putatively produce antimicrobial compounds with broad and narrow-spectrum antibacterial, antiviral, and antifungal activity. The BGCs with potential novelty will be investigated in more detail - in an attempt to link the 'omic' datasets and elucidate the active NPs.

In this study, we demonstrate the value of investigating marine environments and their inhabitants for specialised metabolites with potentially new bioactivities towards drug-resistant pathogens, by integrating classical microbiology and modern '-omics' tools.

A192

Ruthenium metallotherapeutic agents: novel approaches to combatting *Pseudomonas aeruginosa* infections.

Nicole Britten ORCID iD, Jonathan Butler ORCID iD

Manchester Metropolitan University, Manchester, United Kingdom

Abstract

Ruthenium (Ru) metallotherapeutic compounds have potent antimicrobial properties and in contrast to traditional antibiotics, these are thought to elicit antibacterial activity at multiple sites within the bacterial cell, thereby reducing the possibility of resistance evolution. A library of Ru compounds were screened for antimicrobial activity against *Pseudomonas aeruginosa*, with Hexaammineruthenium (III) chloride inhibiting growth of multiple clinical strains of *P. aeruginosa* at ≤32 μg mL^{-1}, with loss of viability occurring within 6 h. Crystal violet biofilm assays showed a decrease in biomass following exposure of *P. aeruginosa* biofilms over a 24 h period. Mechanistic studies demonstrated that the lead candidate targeted the bacterial cell ultrastructure of *P. aeruginosa* as cell perturbations were observed when treated cells were analysed by scanning electron microscopy. Furthermore, exposure of *P. aeruginosa* to Hexaammineruthenium (III) chloride also resulted in a concentration dependent membrane depolarisation, which further supported the antimicrobial mechanistic role. Cell culture *in vitro* scratch assays and 3D skin full thickness wound infection modelling were used to demonstrate the wound healing potential of the lead metallotherapeutic agent. Global changes in gene expression following exposure of *P. aeruginosa* to the lead active were explored by RNA sequencing, with genes involved in ribosome function, cofactor biosynthesis and membrane fusion being downregulated, which provided further insight into the wider mechanisms of antibacterial activity. These findings make a significant contribution towards the search for novel bactericidal agents and further research is now focussed on determining the potential for use as novel adjuvants within medicinal applications.

A193

Investigating ciprofloxacin tolerance in *Pseudomonas* aeruginosa biofilms using transposon directed insertion-site sequencing with expression (TraDIS-Xpress)

Meng Wang, Emma Holden, Muhammad Yasir, Keith Turner, Ian Charles, Mark Webber, Matthew Gilmour

Quadram Institute, Norwich, United Kingdom

Abstract

Pseudomonas aeruginosa is a major opportunistic pathogen which may cause severe infections in immunocompromised individuals, such as patients with cystic fibrosis. Ciprofloxacin, a fluoroquinolone antibiotic, is used extensively to treat *P. aeruginosa* infections but has significantly reduced effectiveness against biofilm-associated *P. aeruginosa*. While many individual genes associated with biofilm formation or ciprofloxacin resistance have been identified, the molecular determinant of biofilm formation relating to *P. aeruginosa* challenged with ciprofloxacin remain incomplete. In this regard, we will employ a transposon-directed insertion-site sequencing with expression (TraDIS-Xpress) approach to systematically investigate the genes contributing to biofilm formation against ciprofloxacin. This improved version of Tn5-based PAO1 transposon library allows investigation of essential genes by influencing expression of the genes surrounding transposon insertions which notably incorporate outward-facing promoters with the transposon. This *P. aeruginosa* transposon library was cultured for 12h, 24h and 48h in 6-well plates containing glass beads as biofilm substrates. Ciprofloxacin was added at 1x MIC with non-ciprofloxacin treatment as a control. Genomic DNA from both the planktonic culture and the biofilm attached on the beads was extracted and sequenced. The pathways and genes related to biofilm formation will be identified by comparing the abundance of insertion sites between the planktonic cells and biofilm cells at each condition. We are currently growing the transposon mutant library at a series of ciprofloxacin concentrations to investigate if those identified biofilm determinants are concentration dependent. In summary, this study design provides the potential to reveal of the molecular mechanism at different stages of biofilm formation and tolerance of *P. aeruginosa* to ciprofloxacin.

A194

ASSESSING ANTIBIOTIC RESISTANCE GENES IN PRISTINE, OIL-CONTAMINATED, AND BIOREMEDIATED SOILS

Olubunmi Evelyn Ajayi[1], Thomas Wagner[1], Julia R de Rezende[1], Obioma Mejeha[2], Leena Kerr[1]

[1]Heriot Watt University, Edinburgh, United Kingdom. [2]Federal University of Technology, Owerri, Owerri, Nigeria

Abstract

Crude oil exploration to meet the growing global energy demand is persistently harming the environment. Bioremediation of crude oil-contaminated soil using organic substrate (biostimulation) has been considered sustainable and cost-effective. However, the choice of biostimulation materials raises concerns about potentially increasing ARGs in treated soils.

Microcosms consisting of soil samples with and without crude oil and amended with manure or compost were set up to investigate the effect of these amendments on ARGs and hydrocarbon degradation. These microcosms were kept at 15°C, and subsamples were taken for DNA extraction and molecular analyses within the first 12 weeks. ARGs are being assessed and compared in soil exposed to oil and manure or compost treatments using PCR with primers targeting specific ARGs of public health importance.

Among the ARGs assessed, *sul1* was detected in the pristine, compost and manure treated soil at the start of the experiment and after 12 weeks. In contrast, *sul2* was absent in pristine soil but present in compost- and manure-treated soil at the start and after 12 weeks. While ARGs, *tetW* and *ermF* were exclusively present in the manure-treated soil at the start and 12 weeks of incubation. However, *tetO* was detected in pristine and manure-treated soil at the start but was absent in all soil samples at week 12.

Further molecular analysis such as metagenomics is required to quantitatively monitor the impact of oil, compost, and manure on ARG pattern and microbial community. Geochemical analysis is being conducted to evaluate hydrocarbon degradation within the samples.

A195

Exploring Nigerian crude oil contaminated sediment microbiomes for antimicrobial peptide (AMP) discovery

Zainab Ahmad Binji, Dr Thomas Thompson, Dr Stephen Kelly, Prof Brendan Gilmore

Queen's University Belfast, Belfast, United Kingdom

Abstract

Antimicrobial resistance (AMR) remains a critical threat, undermining the effectiveness of antibiotics once offered reliable treatment solutions across diverse healthcare settings worldwide. This challenge has mobilized efforts across various disciplines to discover new antibiotics and safeguard those effective for clinical use. This study examines the potential of hydrocarbon-contaminated environments in Nigeria, which may harbor novel antimicrobial compounds to address AMR. Hydrocarbon contamination of previously pristine environments has led to substantial alterations in microbial community compositions, significantly impacting the functional profiles of resident microorganisms. These shift are likely to influence profile of functional bioactive compounds within these communities, including antimicrobial and surfactant peptides, enzymes and secondary metabolites. Antimicrobial peptides (AMPs) are small proteins, fewer than 100 amino acids in length can effectively inhibit or reduce bacterial growth and have garnered significant global research interest as antibiotic alternatives. Fifteen samples of crude oil-polluted sediments from five locations in Nigeria were sampled, metagenomic DNA extraction yielded concentrations between 107 ng/µL to 580 ng/µL, which were subsequently sequenced using the Illumina sequencing platform. Analysis of the metagenomes revealed *Proteobacteria* and *Actinobacteria* as the dominant bacteria. This significant for AMP discovery as both phyla are well-recognized reservoir of bioactive and AMPs. FASTA formatted metagenomic dataset of all samples were converted into protein sequences using EMBOSS followed by analysis with AMP Scanner Vr.2 for AMP prediction. The tool indicated average AMP scores greater than 0.5 and 1 across all five samples locations, validating the hypothesis that oil-contaminated sediments are reservoir of AMP sequenced diversity.

Session : Microbial Physiology, Metabolism and Molecular Biology Forum

A199

“Insights into Mycobacterium abscessus: Exploring Colony Morphotypes, Hydrophobicity, and Transmission Dynamics”

Enass Abdul Kadhum ALMKHADHREE[1], Michael (Prof.) Barer,R.[2], Natalie (Dr.) Garton, J.[2]

[1]Wasit University, Wasit, Iraq. [2]Leicester University, Leicester, United Kingdom

Abstract

Mycobacterium abscessus (Mab), identified in 1953 from a knee abscess (type strain *Mab* ATCC 19977), is an opportunistic pathogen causing severe infections, notably in cystic fibrosis patients with pre-existing lung conditions. While initially thought to originate from environmental sources, recent evidence suggests patient-to-patient transmission. Mab exhibits two colony morphotypes: Smooth (S) and Rough (R), with S strains producing high levels of glycopeptidolipid (GPL) and R strains showing reduced or absent GPL production. The transition from S to R involves mutations abolishing GPL expression, potentially impacting cellular hydrophobicity and aerosol transmission. Comparative analysis of type and clinical S and R variants revealed distinct growth patterns in broth and biofilm assays, with R strains consistently displaying higher hydrophobicity and lacking GPL production. Luciferase-expressing strains were constructed to facilitate investigation of desiccation and UV resistance. No significant differences between S and R were observed in desiccation experiments, while the S strain showed greater resistance to UV exposure. Interestingly, when the two strains were mixed, they showed similar hydrophobicities, possibly indicating redistribution of GPLs in the suspension.

Genome and transcriptome analyses identified numerous polymorphisms associated with R transition, indicating a broader genomic impact than previously recognized. Overall, findings provided limited support for R strains being adapted to airborne transmission, highlighting a wider range of genomic targets involved in the S to R transition. This work extends our knowledge of *Mab* factors linked to pathogenicity and transmission dynamics and emphasizes the complex interplay between colony morphotypes, hydrophobicity, and genetic factors.

A200

The influence of vitamin deficiency on the compositionof the gut microbiota; an in vitro batch culture approach

Amnah Alzaidi, Simon C Andrews, Marie C Lewis, Gemma E Walton, Soon Gweon, Stephen Elmore, Carlos Poveda

University of Reading, Redaing, United Kingdom

Abstract

The gut microbiota can utilise exogenous vitamins, generate vitamins endogenously and release vitamins that support auxotrophs' growth. However, it remains unclear to what degree the gut microbiota is shaped by dietary vitamin availability. This issue is explored by utilising an *in vitro* human batch culture supplied with vitamin-free and supplemented media.

Fresh faecal samples from 10 donors were used to inoculate 48-h batch cultures, two cultures for each donor, one with no vitamins and the other with all vitamins. Samples were collected at 0, 12, 24 and 48 h for analysis by Flow-FISH, SCFA measurement and 16S rRNA gene metagenomics.

Flow-FISH results demonstrated that growth of the microbiota was significantly higher in the presence of vitamins (by ~60% at 12 h; P=0.0017). The absence of vitamins resulted in a greater decline in cell number from 12 to 48 h than seen in the +vit vessels. SCFA levels were ~44% higher at 24 h (P=0.0002) in +vit vessels where a substantial increase was observed in the first 12 h, corresponding to the growth phase. No significant change was found in the α- or β-diversity of the gut microbiota. However, the absence of vitamins caused a decrease in the *Firmicutes*:*Bacteroidota* ratio, from a 'healthy' 1:2 ratio to an 'unhealthy' ratio of 1:8. These findings support previous studies where vitamins supplementation increased the *Firmicutes*:*Bacteroidota* ratio and the production of SCFAs.

Currently, an LC-MS/MS methodology is being developed to monitor production and consumption of each vitamin in the gut models.

A201

Discovering viable therapeutic targets in the causative agent of plague, *Yersinia pestis*

Natasha Turner ORCID iD, Phil Ireland

Dstl, Porton Down, United Kingdom

Abstract

Yersinia pestis is the causative agent of plague. If untreated, plague can have severe consequences in humans, with a case-fatality ratio of 30-100% (CDC., 2024). Antibiotic treatment is currently effective against *Y. pestis*. With antimicrobial resistance (AMR) on the rise, novel antimicrobial therapeutics are needed. The products of bacterial core essential genes are the most promising targets for novel antimicrobials; however, it has been established that some essential proteins are more sensitive to inhibition than others, and should therefore be more viable as therapeutic targets. To establish target vulnerability of these essential genes, we are utilising CRISPR interference (CRISPRi) to assess gene knockdown susceptibility. In this work, we are utilising the Mobile CRISPRi system in *Yersinia pseudotuberculosis* and *Y. pestis*. We have optimised integration of the system into the chromosome and are currently creating a panel of strains in order to generate tuneable expression of essential genes. This research will provide a new insight into viable therapeutic targets and will contribute to the fundamental understanding of this pathogen.

A202

Metals and Host-pathogen Interactions: The Role of Helicobacter pylori Metal Exporter CadA

Haohua Cui ORCID iD, Dennis Linton, Jennifer Cavet

The University of Manchester, Manchester, United Kingdom

Abstract

Helicobacter pylori is a widespread cancer-associated gastric bacterial pathogen, with around 50% of the global population currently infected. Transition metals, such as zinc and copper, are essential for bacterial pathogen survival for being important cofactors for metalloenzymes and metalloproteins. However, these metals can be toxic in excess due to their ability to cause protein mis-metallation. In the human host, both metal essentiality and toxicity are exploited by innate immune defences to control infections via mediating metal sequestration and metal intoxication. To establish chronic infections in the gastric niche, *H. pylori* requires precisely controlled metal homeostasis systems, which include metal-sensor, transporter, metallo-chaperone and storage proteins, making them attractive targets for therapeutic development. However, details regarding these metal-handling systems and their regulation at the host-pathogen interface remain poorly characterised. Our research is centred on identifying and characterising these systems. Here, we have revisited the previously identified metal exporting P-type ATPase, CadA, and showed its crucial role in *H. pylori* zinc homeostasis. Indeed, Δ*cadA* mutants generated in different *H. pylori* wild-type isolates exhibit substantially reduced zinc resistance and over-accumulate cellular zinc. Intriguingly, there is some variation in zinc resistance amongst the different strains, implicating disparity in the presence of other uncharacterised CadA-independent zinc detoxification systems. We have also gained new insights into the function of CopA2 regarding trafficking copper, a second P-type ATPase in *H. pylori*, which remained functionally uncharacterised. Our work regarding the characterisation of CadA, CopA2, and the identification of previously unknown zinc homeostasis systems in *H. pylori* will be described.

A203

Exploring the impact of the stringent response on the structure of ribosomes in Staphylococcus aureus

Orestis Savva[1], Per Bullough[1], Rebecca Corrigan[2]

[1]University of Sheffield, Sheffield, United Kingdom. [2]University College Dublin, Dublin, Ireland

Abstract

The stringent response is conserved across bacterial organisms as a means of survival in response to nutrient deprivation. The response enhances the production of secondary signalling molecules referred to as alarmones, specifically guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp). These alarmones regulate crucial metabolic pathways in the cell, reducing DNA replication, nucleotide synthesis, transcription as well as ribosomal maturation and function. We have previously observed that upon induction of the stringent response in *Staphylococcus aureus*, the formation of 70S ribosomes is inhibited by obstruction of the interactions between ribosomal particles and the family of enzymes RA-GTPases (ribosome associated GTPases); which is essential for maturation and regeneration of ribosomes. These GTPases are conserved throughout prokaryotic organisms and are essential for the maturation of the 30S and 50S ribosomal subunits. (p)ppGpp bind to the GTPases with a higher affinity than GTP, thus preventing association with immature ribosomal subunits and subsequent maturation events. Even though substantial research has been conducted exploring the effects the absence of any of the four individual GTPases has on the ribosomal structure, the role of these enzymes in ribosomal subunit maturation is still unclear, as are the consequences of the inhibition of all four by (p)ppGpp simultaneously. Here, we explore the structure of the 70S ribosomal particles across different *S. aureus* mutants pertaining to the stringent response to further understand the effects of the response in ribosomal maturation and relate those changes to the activities of the RA-GTPases and functionality of the ribosome.

A204

Metabolite Studies in Microbiome Manufacturing: Investigating the metabolism of *Clostridium, Bacteroides,* and *Prevotella* spp. co-cultures to improve probiotic production

Jessica O'Loughlin ORCID iD[1], Karl Burgess ORCID iD[1], Louise Horsfall ORCID iD[1], Catherine Mcnay[2], Sarah Windsor[3]

[1]The University of Edinburgh, Edinburgh, United Kingdom. [2]FUJIFILM Diosynth Biotechnologies, Middlesbrough, United Kingdom. [3]FUJIFILM Diosynth Biotechnologies, Rayleigh, USA

Abstract

The gastrointestinal (GI) microbiome contains many different microbes, notably bacteria, which fulfil numerous and essential biological roles to keep humans healthy and can impact many different health conditions. One way people can manage their GI microbiome composition is by taking probiotics. These are conventionally made using a monoculture-based method, but this has practical and biological limitations. A potential solution and improvement to this process is to use a co-culture manufacturing approach instead. However, large-scale adoption of this is impeded due to a lack of understanding of how each species present interacts when they are grown together. The aim of this project is to use metabolomics to elucidate the interactions between three GI tract-relevant bacterial species (*Bacteroides cellulosilyticus*, *Clostridium beijerinckii*, and *Prevotella herbatica*) in different co-culture set ups to improve our knowledge about how this method could be used for probiotic production. Initial work has focused on modifying the bacterial liquid PYG media to make it free of animal-derived components. This has been successful except for the removal of haemin, which acts as an important iron source for the fastidious *B. cellulosilyticus* and *P. herbatica*, and iron substitutes so far have not worked. Future work aims to investigate whether synergistic interactions between the three species and isolating leghemoglobin from plant tissues could aid growth in animal-free media.

A205

Unveiling the Biofilm Architecture of Pseudomonas aeruginosa in Single and Dual species with E. coli using Multi-Scale Imaging Techniques

Connor MacDonald[1], Beatrice Bottura[2], Gail McConnell[1], Liam Rooney[1]

[1]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom. [2]Cancer Research UK Scotland Institute, Glasgow, United Kingdom

Abstract

Biofilms are complex communities of microbes that pose a significant threat in clinical and industrial settings due to their increased antibiotic resistance, aiding towards recurring chronic infections and contamination of water systems. Previously, intra colony-channels were discovered in mature colony biofilms of *E. coli* and observed to transport nutrients from the surrounding environment. However, most biofilms in nature are polymicrobial and single species biofilms fail to represent the interactions observed in clinical settings. This study used conventional and novel imaging techniques to analyse the internal architecture of single and dual species biofilms of *E. coli* and *P. aeruginosa.* The Mesolens provided a field of view of entire biofilms with a subcellular resolution capturing the differences in macrostructure across the sample area. Innovative specimen preparation methods were designed and implemented to allow the visualisation of the internal structures of *P. aeruginosa* biofilms. Here, we show that *P. aeruginosa* formed radial intra-colony channels and can transport fluorescent microspheres into the biomass, like the channels formed by *E. coli*. Co-cultured *E. coli*/*P. aeruginosa* biofilms developed sinuous channel structures with different morphologies, indicating that *E. coli* may alter the internal architecture of *P. aeruginosa* biofilms. Demonstrating that these channels form and function similarly in two phylogenetically distinct organisms opens avenues for scientific enquiry into other polymicrobial biofilm architectures. These findings of novel internal architecture and interspecies interactions give new insights into polymicrobial biofilms that may assist with the management of biofilm associated infections and offer fresh perspectives on the fight against antimicrobial resistance.

A206

A helping hand: Corynebacterium accolens promotes non-typeable Haemophilus influenzae biofilm formation and survival through metabolic interplay

Jasira Patel[1], Kayleigh Hunwin[1], Mohammed Bhogadia[1], David Cleary ORCID iD[2], Martin Grootveld ORCID iD[1], Raymond Allan ORCID iD[1]

[1]De Montfort University, Leciester, United Kingdom. [2]Universty of Birmingham, Birmingham, United Kingdom

Abstract

Non-typeable *Haemophilus influenzae* (NTHi), a pathobiont residing in the human respiratory tract, is a key contributor to various respiratory tract infections. As antimicrobial resistance in NTHi escalates, there is an urgent need to better understand how it colonises the respiratory mucosa and transitions towards infection, thereby guiding the development of future treatment strategies. *Corynebacterium accolens*, a respiratory commensal, exhibits antimicrobial activity against other respiratory pathogens including *Streptococcus pneumoniae* and *Staphylococcus aureus*, however, its interaction with NTHi remains unexplored. To investigate this, we established a co-culture biofilm model to examine the metabolic interplay between *C. accolens* and NTHi, hypothesising that *C. accolens* influences NTHi biofilm formation through the breakdown or production of specific metabolites. Co-culture biofilms were grown in chemically defined media for 24 and 48 hours, viability assessed through colony-forming unit enumeration, biofilm structure and spatial organisation evaluated through confocal microscopy, and metabolic interplay assessed using 1H NMR spectroscopy. Our results revealed that *C. accolens* and NTHi coexist within the same biofilm environment, with CFU counts indicating a significant increase in NTHi, and confocal imaging confirming coexistence and distinct spatial organisation within biofilms. Metabolic profiling via 1H NMR showed distinct shifts in metabolic activity, with various carbohydrates, fatty acids, and amino acids potentially contributing towards a symbiotic relationship that benefits NTHi. The development of this novel commensal-pathogen co-culture model has provided new insight into interactions that potentially support NTHi persistence in the respiratory tract, whilst also furthering our understanding of the role of commensals in multispecies environments.

A207

Activity of individual drugs against biofilm in hypervirulent Klebsiella pneumoniae

Alimire· Aimaiti[1], Qingqing Xu[1,2]

[1]Institute of Antibiotics, Huashan Hospital, Fudan University, Shanghai, China. [2]Department of Biochemistry, Cambridge, United Kingdom

Abstract

Biofilm formation is one of the important virulence traits in *Klebsiella pneumoniae*. The objective of this study was to investigate the antibiofilm activities of ceftazidime, piperacillin/tazobactam and amikacin against hypervirulent *K. pneumoniae* (hvKP). In vitro antibacterial susceptibility showed that 13 hvKP clinical isolates were susceptible to ceftazidime, piperacillin/tazobactam and amikacin. Amongst, eight strains (61.5%) showed strong biofilm formation, while three strains displayed moderate biofilm formation, and two strains demonstrated weak biofilm formation. Further two strong biofilm-forming strains, NTUH-K2044 and KP20-350, were subjected to biofilm inhibition and eradication assays to evaluate the antibiofilm activity of the aforesaid drugs. Half-fold minimum inhibitory concentration (1/2 × MIC) of ceftazidime, 1 × MIC of piperacillin/tazobactam and 1 × MIC of amikacin are sufficient for biofilm inhibition activities against NTUH-K2044 and KP20-350. While biofilm eradication concentrations of ceftazidime, piperacillin/tazobactam and amikacin are 1 × MIC, 1 × MIC and 4 × MIC, respectively. In summary, ceftazidime, piperacillin/tazobactam and amikacin served as the first-line antibacterial agents, exhibited effective biofilm inhibition and eradication activities against hvKP strains.

A208

Post-translational regulation of glucose metabolism in *Streptomyces*

Kaisha Murray, Daniel Larcombe, Iain Hunter, Paul Hoskisson

University of Strathclyde, Glasgow, United Kingdom

Abstract

Streptomyces are prolific producers of bioactive specialised metabolites which are biosynthesised from primary metabolic building blocks. Understanding the regulation of primary metabolism will enable engineering of metabolism to increase production of antibiotics and other clinically important metabolites from *Streptomyces*. One recently emerged regulatory mechanism in *Streptomyces* is post-translational modification (PTM) by crotonylation. The role of crotonylation as a post-translational modifier of glucose-kinase (Glk) and carbon metabolite repression (CCR) in *Streptomyces* metabolism is poorly understood. A previous study suggested the importance of crotonylation in the regulation of CCR in *Streptomyces roseosporus,* however, no evidence currently exists to suggest this mechanism occurs widely in *Streptomyces* species. Here we show that introducing the putative crotonylation and decrotonylation machinery from *Streptomyces coelicolor* into the industrial species *Streptomyces clavuligerus* alters antibiotic yield. We found that an over-expression of each of the components of the crotonylation/decrotonylation machinery resulted in a 1.5-fold increase in the yield of clavulanic acid. Furthermore, the role crotonylation plays in PTM of *Streptomyces* metabolism was also determined in relation to the regulation of CCR in *S. coelicolor* and *S. clavuligerus* through knockout mutants and targeted metabolomics. This will allow for an enhanced understanding of the complex control mechanisms involved in *Streptomyces* primary and specialised metabolite production. Through this understanding there is potential to increase the yield of these vital natural products in industrial strains and make steps towards the identification and development of new clinically useful bioactive metabolites.

A209

Fluorescent Drugs for Difficult Bugs: Quantitative Imaging of Fluorescent Antibiotics to Track Antibiotic Uptake and Resistance in Biofilms

Ash Eana ORCID iD, Gail McConnell ORCID iD, Liam M. Rooney ORCID iD

Strathclyde Institute for Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom

Abstract

Biofilms are aggregated communities of microbes bound by a self-secreted polymeric extracellular matrix. They remain a strategic priority for public health due to their intractability, chronicity and ability to potentiate antimicrobial resistance (AMR). Networks of complex nutrient transport channels have recently been reported in *Escherichia coli* biofilms. However, their potential to transport antimicrobial agents remained unclear. Moreover, their effect on drug trafficking dynamics, the emergence of resistant or tolerant sub-populations, and their role in potentiating cell death remained unclear due to the difficulty in studying these aspects across large spatial scales with high resolution. In this study, we show that non-viable cells accumulate in *E. coli* biofilm channels following antimicrobial treatment, and that channel-mediated antibiotic uptake is possible in these large biofilms. Using advanced imaging techniques, intensity-based image analysis, and a modified version of the Kirby-Bauer method, we quantified cell death and antibiotic uptake *in situ*, showing a significant increase in cell death following treatment with colistin. We then directly visualised the trafficking of BOCILLIN-FL, a fluorescent penicillin conjugate, through the nutrient transport channels of HcRed-1-expressing *E. coli* biofilms. Bacterial cells compromised by the bactericidal activity of BOCILLIN-FL were stained using a fluorescent viability dye to visualise and then quantify localised cell death correlated with the transport of fluorescent antibiotics. This study provides new insight into the channel-mediated uptake of antibiotics in biofilms at unprecedented spatial scales and demonstrates the potential to exploit this process to lower the burden of AMR with new exploitative antimicrobial delivery methods.

A210

Characterization of metal-homeostasis systems in the human gastric pathogen *Helicobacter pylori*

Ivan Y. L. Chan ORCID iD[1], Dennis Linton[1], Jennifer S. Cavet[2]

[1]University of Manchester, Manchester, United Kingdom. [2]Manchester, Manchester, United Kingdom

Abstract

Transition metals are indispensable for all aspects of metabolism as they are required for approximately a third of the proteome, yet are toxic in excess. During infection, the host innate immune defences exploit these properties and manipulate metal levels to attack invading microbes via metal-deprivation and/or metal intoxication. In response, microbes employ various strategies to acquire sufficient of each metal to meet the metal demands of their proteins whilst avoiding metal-poisoning. In many bacterial pathogens, the metal homeostasis systems represent crucial virulence factors for successful host colonization and infection. However, the mechanisms employed by the human gastric bacterial pathogen, *Helicobacter pylori*, to sense and respond to fluctuating metal levels in the stomach, remain largely uncharacterized. A better understanding of these systems in *H. pylori* will potentially inform the development of novel anti-*Helicobacter* therapeutic approaches. Our research is focused on the characterization of the *H. pylori* metal resistance systems with recent work examining the role of the resistance-nodulation-cell division exporter CznABC which was previously described as a zinc exporter and associated with urease activity (Stähler *et al.*, 2006). We have shown that CznABC provides an additional level of defence against high zinc toxicity alongside the zinc-exporting P_{1B}-type ATPase CadA which acts as the primary zinc exporter. We uncovered new additional insights regarding the function of CznABC with respect to metal export and resistance, the regulation of intracellular metal ratios and the oxidative stress defences in *H. pylori*. Our work regarding the characterization of CznABC in *H. pylori* will be described.

A211

Characterization of metal-homeostasis systems in the human gastric pathogen *Helicobacter pylori*

Ivan Y. L. Chan ORCID iD, Dennis Linton, Jennifer S. Cavet

University of Manchester, Manchester, United Kingdom

Abstract

Transition metals are indispensable for all aspects of metabolism as they are required for approximately a third of the proteome, yet are toxic in excess. During infection, the host innate immune defences exploit these properties and manipulate metal levels to attack invading microbes via metal-deprivation and/or metal intoxication. In response, microbes employ various strategies to acquire sufficient of each metal to meet the metal demands of their proteins whilst avoiding metal-poisoning. In many bacterial pathogens, the metal homeostasis systems represent crucial virulence factors for successful host colonization and infection. However, the mechanisms employed by the human gastric bacterial pathogen, *Helicobacter pylori*, to sense and respond to fluctuating metal levels in the stomach, remain largely uncharacterized. A better understanding of these systems in *H. pylori* will potentially inform the development of novel anti-*Helicobacter* therapeutic approaches. Our research is focused on the characterization of the *H. pylori* metal resistance systems with recent work examining the role of the resistance-nodulation-cell division exporter CznABC which was previously described as a zinc exporter and associated with urease activity (Stähler *et al.*, 2006). We have shown that CznABC provides an additional level of defence against high zinc toxicity alongside the zinc-exporting P_{1B}-type ATPase CadA which acts as the primary zinc exporter. We uncovered new additional insights regarding the function of CznABC with respect to metal export and resistance, the regulation of intracellular metal ratios and the oxidative stress defences in *H. pylori*. Our work regarding the characterization of CznABC in *H. pylori* will be described.

A212

Revealing the Ultrastructure of Live *Candida albicans* Through Super-Resolved Optical Microscopy

Katherine Baxter ORCID iD, Shannan Foylan ORCID iD, Liam Rooney ORCID iD, Gwyn Gould ORCID iD, Gail McConnell ORCID iD

University of Strathclyde, Glasgow, United Kingdom

Abstract

Candida albicans, a common fungal pathogen, is known for its ability to switch between yeast form and hyphal form, aiding its survival and pathogenicity within the human host. While standard light microscopy has provided valuable insights into its cellular structure, the limitations of traditional optical resolution have left much of its ultrastructure uncharted. Here, we report the first stimulated emission depletion (STED) microscopy of *C. albicans*, producing super-resolved optical images with unprecedented levels of detail, capturing structural nuances that are essential for understanding its growth, morphogenesis, and interaction with host cells.

We have developed a robust protocol for live cell staining of *C. albicans* that is compatible with the conditions needed for STED microscopy, which allows visualisation of dynamic cellular components, including organelle distribution and cell wall remodelling. These methods enable us to resolve features smaller than the diffraction limit of conventional light microscopy, potentially offering new insights into the ultrastructural adaptations of *C. albicans* under various environmental conditions.

Our study indicates the potential power of super-resolution imaging in fungal research, providing a clearer picture of the cellular machinery of *C. albicans* and advancing our understanding of the role of this important pathogen in human health and disease.

A213

Xenosiderophore from a co-infecting pathogen promotes *Staphylococcus aureus* fitness in iron limited conditions.

Charlotte Jeffery, Christian Jenul

University of Leicester, Leicester, United Kingdom

Abstract

Iron is essential to bacterial life, but access is restricted during infection, and so bacteria must scavenge iron from the host environment. One method is through the biosynthesis of siderophores. *S. aureus* produces two siderophores (staphyloferrin A and B) and is also able to use siderophores produced by other microorganisms (xenosiderophores). In particular, *S. aureus* uses the Fhu system to take up hydroxamate xenosiderophores. Cystic Fibrosis (CF) infections are polymicrobial in nature and *S. aureus* as well as *B. cenocepacia* are prominent bacterial pathogens infecting the lungs of CF patients. Ornibactin is a hydroxamate siderophore produced by *B. cenocepacia* that has not previously been shown to be utilised by *S. aureus*.

Using *B. cenocepacia* H111 mutants lacking pyochelin, ornibactin, or both, and a *S. aureus* LAC mutant lacking both staphyloferrins, we tested whether ornibactin could substitute for the lack of endogenously produced siderophores.

Cell-free supernatants from *B. cenocepacia* cultures containing ornibactin supported the growth of the *S. aureus* staphyloferrin mutant under iron limited conditions. This growth increase did not occur with supernatants lacking ornibactin. Additionally, no growth increase was observed for a *S. aureus* staphyloferrin mutant lacking the xenosiderophore receptor FhuD2 in the presence of ornibactin.

This work shows *S. aureus* can utilise ornibactin from *B. cenocepacia* for iron uptake, indicating an as of yet uncharacterized interaction between *S. aureus* and *B. cenocepacia*. The inability of a *S. aureus* staphyloferrin mutant lacking FhuD2 to utilize ornibactin as an iron source indicates that the Fhu system is involved in ornibactin uptake.

A214

The impact of air pollution on *Staphylococcus aureus*

Chloë Wellings ORCID iD, J Purves, JM Ketley, JA Morrissey

University of Leicester, Leicester, United Kingdom

Abstract

According to the World Health Organisation, air pollution from fine particular matter ($PM_{2.5}$) caused 4.2 million premature deaths worldwide in 2019. $PM_{2.5}$ is released as a result of industrial and vehicle emissions, as well as brake and tyre wear. It has been shown that exposure to $PM_{2.5}$, of which black carbon (BC) is a major component, not only has harmful effects on human respiratory systems, but also alters the behaviour of the bacteria that colonise us. In our recent publications we have shown that *Staphylococcus aureus* has an increased ability to adhere to and invade respiratory epithelial cells after exposure to and co-exposure with BC, as well as increased colonisation of the murine respiratory tract *in vivo*, and alterations to biofilm antibiotic tolerance. We have shown BC directly influences the differential expression of a range of *S. aureus* genes, including those associated with toxin production and immune evasion (such as *hla*, *chp*, *psmα* and *β*). Our current work involves understanding the mechanisms behind this response through the physical and chemical impact BC has on *S. aureus.* The genetic mechanisms behind these effects and the BC-induced phenotypic changes to bacteria are being explored, along with its potential regulators.

A215

Colicin V plasmids transfer to genetically diverse Avian Pathogenic E. coli (APEC) lineages and influence nutrient metabolism

Charlotte Birdsall, Jai Mehat, Roberto La Ragione

University of Surrey, Guildford, United Kingdom

Abstract

Plasmids that confer beneficial traits, such as antimicrobial resistance genes (AMR) and virulence factors, are often maintained in bacterial species and communities. These genetic elements are frequently mobile, and can transfer within the community, often providing a selective fitness advantage to their hosts. Within Avian Pathogenic *E. coli* (APEC), the leading cause of bacterial extra-intestinal poultry disease, ColV plasmids are prevalent across the diverse pathotype and are associated with extra-intestinal survival and pathogenicity.

However, the drivers that influence plasmid dissemination within the gut community, and that underpin the capability of APEC to proliferate and compete within the intestinal reservoir, are unknown.

We identified a 155kb, conjugative ColV/IncF plasmid (pCB001) harboured by an ST-117 APEC isolate, encoding multiple virulence genes and a multi-drug resistance (MDR) integron. pCB001 was shown to be promiscuous, and readily transferred to phylogenetically distant lineages of *E. coli*, facilitating the spread of virulence genes and MDR to co-resident *E. coli* in the gut. Generation of a plasmid-cured ST-117 isolate revealed pCB001 enhances growth under nutrient-limited conditions and confers increased iron sequestration. The pCB001-free strain also displayed reduced capability to metabolise 8 carbon-sources including melibiose, despite the lack of catabolic genes on the plasmid. Given whole genome sequencing of the cured isolate identified no mutations in catabolic genes, we hypothesise that ColV plasmids in APEC influence carbon metabolism at the transcriptomic level, thereby regulating bacterial fitness. Future work will investigate the fitness of plasmid-harbouring APEC within the gut microbiota to understand the factors influencing carriage and dissemination.

A216

Cystic fibrosis airway-associated pathogens display strong signatures of genetic adaptation during growth in artificial sputum media

Jemima Swain, Martin Welch

University of Cambridge, Cambridge, United Kingdom

Abstract

The airways of people with cystic fibrosis (pwCF) are often colonised by a “zoo” of microbes, including Gram-negative and Gram-positive bacteria, and fungi. These polymicrobial lung infections remain a leading cause of morbidity and mortality in pwCF. Recent years have seen the development of numerous *in vitro* models to study CF pathogens. These typically favour the use of host-mimicking culture media, such as artificial sputum medium (ASM), with the aim of capturing physiologically-relevant phenotypes. However, most “domesticated” laboratory strains have long-since become adapted to growth in common laboratory media (typically, lysogeny broth (LB)), and so experience a strong selective pressure when they encounter more “physiologically-relevant” media. This leads to an under-appreciated problem in artificial laboratory evolution (ALE) studies where more overt selection pressures are applied (e.g., challenge with a stressor): which, if any, of the observed genetic changes reflect adaptation to the medium, and which reflect adaptation to the intended selection pressure? This problem is rarely acknowledged or addressed in the literature. Therefore, in this study, we examined the temporally-resolved mutational dynamics of several CF-associated species, including both “domesticated” laboratory strains and clinical isolates, when cultured in ASM. To do this, each strain was passaged through 30 distinct “evolutionary bottlenecks”. Strains were analysed by whole-genome sequencing at the midpoint and endpoint, and compared with the “input” samples used to inoculate at the start. The data reveal clear signatures of genetic adaptation in both laboratory and clinical isolates, and furthermore, that these adaptations have major fitness consequences for the strains.

A217

Unravelling nitrogen regulation networks of the enteric pathogen *Campylobacter jejuni* by integration of Transposon Directed Insertion-site Sequencing (TraDIS) into a continuous culture model of nitrogen limitation

Aidan Taylor[1], Jack Whitmore[1], Emily Stoakes[2], Andrew Grant[2]

[1]University of Reading, Reading, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom

Abstract

Campylobacter jejuni is a zoonotic, foodborne pathogen and the leading cause of human bacterial gastroenteritis worldwide. At a cost of £500 million/annum and >300,000 predicted cases/annum in the UK alone, *Campylobacter* infections represent a significant economic and health burden. *C. jejuni* is a highly specialised, host dependent pathogen with a small genome, and as such has a limited metabolic repertoire in several respects. Nitrogen assimilation is one example: *C. jejuni* is limited to just 4 amino acids as nitrogen source and, unlike most model bacteria, is unable to utilise exogenous ammonium. *C. jejuni* also lacks almost all the canonical nitrogen regulation features of *E. coli*, including the PII / Ntr system. How *C. jejuni* regulates its nitrogen pool and thrives, despite this apparently harsh nutrient limitation, is unclear.

We are utilising the recent development of Transposon Directed Insertion-site Sequencing (TraDIS) for *Campylobacter* spp. by integration into a continuous culture model of nitrogen limitation. TraDIS can be used to extrapolate the essentiality of genes under a given condition by the comparative survival of comprehensive transposon (Tn)-insertion mutant libraries. We have fed Tn libraries into our continuous culture model and comparative survival quantified between steady states of nitrogen-limited vs. replete conditions. To our knowledge, this is the first reported marriage of TraDIS with continuous culture. Here, we present our key findings from this unique technique and how it has informed our understanding of nitrogen regulation in this critical human pathogen.

A218

The Effects Of Copper And Histatins In Oral *Neisseria*.

Archie Howell[1], Safa Chougule[1], Nick Jakubovics[2], Janet Quinn[2], Karrera Djoko[1]

[1]Durham University, Durham, United Kingdom. [2]Newcastle University, Newcastle, United Kingdom

Abstract

The human oral cavity hosts a diverse community of bacteria. These oral bacteria contribute to oral and systemic health. The mechanisms mediating oral bacterial homeostasis are poorly understood but proteins, peptides, metals and other small molecules are likely to play a role. Oral bacteria are exposed to metals such as copper (Cu) from food and saliva that act as a microbial nutrient and toxin. Human saliva also contains a family of peptides called histatins that can bind Cu ions and exhibit antimicrobial properties against oral fungus like *Candida albicans.* However, the effects of histatins and Cu against oral bacteria are unknown.

So far, we have developed a chemically defined medium that supports growth of several oral bacteria, including *Neisseria subflava*. Moderate Cu supplementation promoted the growth of *N. subflava,* but high Cu induced toxicity. Measurement of cellular Cu by ICP-MS showed a positive correlation between Cu treatment and intracellular accumulation. Analysis of gene expression by qRT-PCR showed Cu-dependent changes in the transcription of genes that encode enzymes important in aerobic respiration and denitrification. We also found that histatins promote growth and survival of *N. subflava* with and without Cu. However, these effects were influenced by how the bacterial inoculum was prepared, suggesting *N. subflava* physiology influences interactions with Cu and Histatins.

We are currently investigating the biochemical mechanisms that underpin these findings. The results will provide insights into how salivary copper and histatins affect *N. subflava* physiology, and thus influence the health of the oral microbiome.

A219

Epigenetic control of replication, gene expression and growth phase by ADP-ribosylation of DNA in Mycobacterium tuberculosis

Rachel Butler[1], Marion Schuller[2], Ritu Jaiswal[3], Jayanta Mukhopadhyay[3], Jim Barber[1], Emily Wasson[1], Alex Couto Alves[1], Ivan Ahel[2], Graham Stewart[1]

[1]University of Surrey, Guildford, United Kingdom. [2]University of Oxford, Oxford, United Kingdom. [3]Bose Institute, Kolkata, India

Abstract

Mycobacterium tuberculosis maintains long-term infections characterised by the need to regulate growth and adapt to contrasting in vivo environments. Here we show that M. tuberculosis complex bacteria utilise reversible ADP-ribosylation of single-stranded DNA as an epigenetic mechanism to coordinate stationary phase growth with transcriptional adaptation. The DNA-modification is controlled by DarT, an ADP-ribosyl transferase, which adds ADP-ribose to thymidine, and DarG, which removes the base modification. Using darG-knockdown M. bovis BCG and affinity purification of ADP-ribosylated genomic DNA, we map the first DNA-ADP-ribosylome from any organism. We show that inhibition of replication by DarT is reversible and accompanied by extensive ADP-ribosylation at the origin of replication (OriC). In addition, we observe ADP-ribosylation across the genome and use in vitro transcription to demonstrate that ADP-ribose-thymidine alters the transcriptional activity of M. tuberculosis RNA polymerase. Furthermore, we demonstrate that during stationary phase, DarT-dependent ADP-ribosylation of M. tuberculosis DNA modulates the interaction of transcription regulators and is required to optimally induce expression of specific molecular systems important for adaptation to stationary phase “growth”. In summary, we will describe how ADP-ribosylation of DNA provides a coordinating epigenetic link through every aspect of DNA biology from replication to transcription to translation.

A220

Investigating the molecular mechanisms underpinning mycovirus-mediated phenotypes in filamentous fungi

Josephine Battersby[1], Harry Aston[2], Ioly Kotta-Loizou[1,2], Robert Coutts[1]

[1]University of Hertfordshire, Hatfield, United Kingdom. [2]Imperial College London, London, United Kingdom

Abstract

Aspergillus fumigatus, the primary agent of invasive aspergillosis infections (IAIs), harbours numerous mycoviruses, several of which modify host physiology, including changes to mycotoxin production, virulence, and stress tolerance. Aspergillus fumigatus polymycovirus 1 (AfuPmV1) is a dsRNA virus from the *Polymycoviridae* family comprising four mono-cistronic segments. AfuPmV1 was found to reduce the virulence of IAI in a mammalian model, alluding to potential therapeutic applications of this virus. With global cases of IAI's rising amid emerging antifungal resistance, this finding could have ramifications in medicine. To fulfil the potential application of mycoviruses, the currently poorly understood molecular mechanisms underpinning virus-mediated phenotype modulation require delineation. Our aim is to outline these mechanisms by investigating AfuPmV1 protein biochemistry, uncovering the fungal host components they interact with, and the pathways implicated. We have constructed cassettes for expression of affinity tagged AfuPmV1 proteins from (i) the *A. fumigatus* genome using CRISPR-Cas9 genome engineering, and (ii) using the *Pichia pastoris* expression system. Expression *via* both approaches will be proceeded by downstream protein interaction assays to explore interacting fungal host components. Phenotypic assays will then be conducted on the *A. fumigatus* strains overexpressing individual viral proteins, exploring alterations to virulence and antifungal drug resistance. Expression constructs with fluorescently tagged viral proteins will also be assembled, facilitating protein subcellular localisation by fluorescence microscopy. Insight from these experiments will illustrate how mycoviruses interact with their host to change its physiology, potentially enabling manipulation of viral genomes to induce user-defined host phenotypes in the future.

A221

Pantothenate of the Opera: Unmasking the Transporter of Vitamin B5 in Epsilon-proteobacteria

Jack Whitmore[1], Emily Stoakes[2], Andrew Grant[2], Aidan Taylor[1]

[1]University of Reading, Reading, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom

Abstract

There is no known transporter for vitamin B5 (pantothenate) in Epsilon-proteobacteria, including *Campylobacter*, the leading cause of human bacterial gastroenteritis worldwide. Vitamin B5 is an essential precursor for the ubiquitous cofactor Coenzyme A.

Genome-wide association and phylogenetic analysis highlight that a major driving factor for efficient host adaptation in *Campylobacter* is the presence or absence of the *panBCD* genes that encode the vitamin B5 biosynthesis pathway. These genes are almost always present in Campylobacter isolated from cattle; however, they are typically absent in isolates from chickens and wild birds. This demonstrates that even though many strains can biosynthesise vitamin B5, others, particularly isolates from chickens, are auxotrophs and require the transport of exogenous vitamin B5 into the cell: a transporter must therefore exist.

To identify this putative vitamin B5 transporter, we created a *Campylobacter jejuni* panBCD mutant, a vitamin B5 auxotroph, meaning its growth is entirely dependent on supply and uptake of exogenous vitamin B5. We introduced this *panBCD* mutant into a vitamin B5-limited continuous culture; once steady state was attained, we spiked the culture with an excess of vitamin B5, anticipating this would downregulate and therefore reveal the transporter gene through comparative RNA sequencing. We discovered, interestingly, that this transporter is not transcriptionally regulated. We therefore sought alternative methods for its discovery.

Using newly established Transposon Directed Insertion-Site Sequencing (TraDIS) methods for Campylobacter, we generated a comprehensive vitamin B5 auxotroph transposon mutant library, unmasking the identity of this elusive transporter in Epsilon-proteobacteria.

A222

Biological metal recovery from printed circuit boards under low pressure for potential application in space habitation

Yeongmi Park ORCID iD, Min-Sang Kim, Uijeong Han, Si-Kyung Cho ORCID iD

Dongguk University, Goyang, Korea, Republic of

Abstract

Space exploration and further habitation beyond Earth have been thrivingly studied. Especially for long-duration space missions, resources should be supplied steadily, as they determine the health of the crew as well as the performance of the mission. Biological resource recovery, such as bioleaching, could be a breakthrough in extraterrestrial conditions both in environmental and economic aspects. One of the factors that could affect to space bioleaching during operation and final yield is low pressure. The technical feasibility of biological resource recovery under low pressure is still unclear; however, it is anticipated that it could reduce the engineering burden of constructing the reactor system. In this study, the recovery rate of metals from spent printed circuit boards (PCBs) under low pressure (less than 1 bar) was evaluated. The inlet gas, composed of oxygen and nitrogen, was injected with a different pressure, altering the gas composition. The yield of metal recovery was compared to the control (1 bar with 79% nitrogen and 21% oxygen) under different conditions. After the experiment, the viability of microbes was evaluated using qPCR. This study is anticipated to provide more detailed insights into further space habitation research.

A223

Alternative ribosome expression in *Mycobacterium bovis* BCG and antibiotic tolerance - separating causality from concurrence

Rachel Butler ORCID iD, Jim Barber, Graham Stewart

University of Surrey, Guildford, United Kingdom

Abstract

Mycobacterium tuberculosis (MTB) and other members of the MTB-complex are obligate pathogens of mammals. Their unique life and transmission cycle finds them in micro-environments with extremes in concentration of zinc. In the macrophage, zinc is pumped into the phagosome by the host cell causing zinc poisoning. However once MTB exits from the phagosome and macrophage, the extracellular milieu and caseum are zinc restricted due to zinc sequestration by the neutrophil protein calprotectin. To counter low zinc levels, MTB expresses genes that are members of the "Zur" regulon. These include zinc uptake transporters, and four "alternative" ribosome paralogues that are zinc-independent. Under zinc replete conditions, Zur is bound to zinc and acts as a transcriptional repressor. During zinc limiting conditions, Zur dissociates allowing transcription of the Zur regulon including the alternative ribosomes. The expression of alternative ribosomes by mycobacteria is of considerable interest due to the association of expression with antibiotic tolerance.

In this study, we have created a fluorescent reporter construct to measure alternative ribosome promoter activity under a range of culture conditions and a range of laboratory media. Our data show two scenarios under which the alternative ribosome promoter is active – under zinc restriction and in late stationary phase. We tested antibiotic tolerance in these populations to determine whether alternative ribosome expression is the cause of antibiotic tolerance, or whether expression is concurrent with the appearance of antibiotic tolerance due other changes in physiological state.

A224

Regulation and Biosynthesis of Chloramphenicol in *Streptomyces venezuelae*

Neil Holmes ORCID iD[1], Rebecca Devine ORCID iD[1], Nicolle Som ORCID iD[2], Ainsley Beaton ORCID iD[1], Martin Rejzek ORCID iD[1], Barrie Wilkinson ORCID iD[1], Matthew Hutchings ORCID iD[1]

[1]Department of Molecular Microbiology, John Innes Centre, Norwich Research Park, Norwich, United Kingdom. [2]Quadram Institute, Norwich Research Park, Norwich, United Kingdom

Abstract

Streptomyces venezuelae is the native producer of chloramphenicol (Ehrlic *et al.,* 1948) and is a genetic model for the study of *Streptomyces* bacteria. The study of the regulation and biosynthesis of chloramphenicol represents an opportunity to obtain a complete understanding of how *Streptomyces* bacteria control both the temporal and spatial dynamics of natural product synthesis.

The regulation of chloramphenicol biosynthesis is controlled by at least two transcriptional regulators. The cluster situated regulator CmlR (Fernández-Martínez *et al.,* 2014) positively activates transcription of the biosynthetic genes while the pleiotropic regulator MtrA (Som *et al.,* 2017) binds upstream of *cmlR* and between the genes *cmlN/F* that encode transporter proteins. Deletion of *mtrA* switches on constitutive chloramphenicol production, causing a huge increase in production of the biosynthetic enzymes and the antibiotic.

Chloramphenicol is derived from the shikimate pathway which normally leads to production of aromatic amino acids via chorismic acid (Vining & Stuttard., 1995). Halogenation of chloramphenicol precursors occurs at the later stages of biosynthesis to produce the final active molecule. We deleted the genes proposed to be involved in the halogenation and transfer of the chlorinated part of the molecule to enable chloramphenicol production and the *mtrA* mutant proved to be a useful background to look at the accumulation of the metabolites that were produced instead of the final chloramphenicol molecule. Here we present our progress in understanding MtrA regulation of the biosynthetic gene cluster and control of the final stages of chloramphenicol biosynthesis of this important antibiotic.

A225

Structural Insights into the Oligomerization and Conformational Transitions of Isocitrate Lyase in Pseudomonas aeruginosa

Prasanthi Medarametla ORCID iD[1,2], Paul Brear[2], Steven W Hardwick[2], Dimitri Y Chirgadze[2], Martin Welch[2]

[1]University of Eastern Finland, Kuopio, Finland. [2]University of Cambridge, Cambridge, United Kingdom

Abstract

Isocitrate lyase (ICL) plays a crucial role in central carbon metabolism, particularly in the glyoxylate shunt pathway, which enables pathogens like *Pseudomonas aeruginosa* to persist during infections by redirecting metabolic flux from the TCA cycle. This is vital for the pathogen's survival and virulence, making ICL an attractive target for drug development. However, the structural details of ICL from *P. aeruginosa* remain limited. Here, we investigated the structural basis of ICL function using X-ray crystallography, cryo-EM, and molecular dynamics simulations. Our studies reveal how binding of allosteric regulators, including pyruvate, and oxaloacetate, induces conformational changes that regulate the enzyme's activity. Specifically, we observed the transition of the catalytic loop from an open to a closed state upon regulator binding, which also influences ICL oligomerization. These findings provide new insights into the mechanisms of ICL regulation and its potential as a drug target.

A226

Exploring the Impact of Microbial Communities on the Cultivation of Microalgae

Hongli Wang ORCID iD, Alexandra Gimblett

Swansea University, Swansea, United Kingdom

Abstract

Microalgae play a crucial role in the global primary production and harbour great potential for various biotechnological applications. However, the industrialization of microalgae is limited by several factors, such as the need to improve their robustness in large open systems, enhance their immune response to external species, and increase their biomass yield. Addressing these challenges is crucial for the successful commercialization of microalgae. Algae-associated bacteria often referred to as the "second genome" of algae, play a crucial role in various stages of the algal life cycle, including growth, immunity, and adaptive evolution. As a result, it is essential to identify advantageous bacteria that can enhance algal growth and investigate the mechanisms of bacterial-algal symbiosis to increase the bioenergy yield of microalgae. In this study, bacteria isolated from the green microalgae *Tetradesmus obliquus* were co-cultured to construct a co-culture model system. The mechanism of symbiosis between the beneficial bacteria and microalgae will be elucidated through comparative metabolomics and metatranscriptomics. We want to explore the effect of nutrient availability (nitrogen, phosphorus) on the interaction with the overall aim to employ these co cultures on nutrient rich waste water for a green bioeconomy of microalgae. The main objective of this study was to analyse microalgal microbiomes cultivated from wastewater and identify potentially beneficial bacteria within these microbiomes with the aim to make microalgal wastewater treatment systems viable at large scales.

A227

Characterisation of a Zinc-Finger Protein from the Human Bacterial Pathogen *Campylobacter jejuni*

Manar Alawkar ORCID iD, Evita Mayasari, Jennifer Cavet ORCID iD, Dennis Linton ORCID iD

University of Manchester, Manchester, United Kingdom

Abstract

Campylobacter jejuni is annually responsible for more than 700,000 gastrointestinal infections resulting in 22,000 hospitalisations and 100 deaths in the UK. Zinc is required for enzyme catalytic activity, protein stabilisation, and folding. Zinc finger (ZF) proteins contain a structural zinc-binding motif with one or more zinc ions attaching to different arrangements of histidine and/or cysteine residues. ZF proteins perform diverse roles with prokaryotic ZF proteins having been assigned multiple functions including in virulence, motility, transcriptional regulation, symbiosis, production of surface components and metal homeostasis. *C. jejuni* Cj1164c is a 10.2 kDa Cys4-type ZF protein of unknown function and we have shown that it irreversibly binds one zinc ion at its amino-terminal zinc-finger motif and possesses a second exchangeable metal-binding site at its carboxy-terminus. To functionally characterise this protein, a *C. jejuni* strain with an inactivated *cj1164c* gene was generated and tolerance to a range of environmental stresses examined. Notably, the *cj1164c* mutant was found to have reduced tolerance to reactive oxygen species (ROS), including hydrogen peroxide and the ROS generator menadione sodium bisulfite, but not other tested stresses including elevated metal levels. These data are consistent with a role for Cj1164c in ROS resistance. These findings add to our understanding of how this pathogen evades antimicrobial treatments in the food chain and host immune defences to cause infections, thus providing insight for the development of targeted *campylobacter* control strategies and treatments. Our work regarding the further characterisation of the function and regulation of *cj1164c* will be presented.

A228

The antibacterial efficacy of Compound X: differing iron restriction mechanisms between Gram-negative and Gram-positive bacteria

Lauren Jordan, Joy Paterson, Gary Sharples ORCID iD

Durham University, Durham, United Kingdom

Abstract

Metals are estimated to serve as structural and catalytic cofactors in half of all proteins, making them essential for all forms of life. Vertebrates have capitalised on this necessity for metals by evolving the means to both sequester and deliver excess metals to prevent pathogen proliferation, a system termed nutritional immunity. Synthetic metal chelating agents can mimic these innate immune processes by binding metal cations with high affinity. One example is Compound X, which is incorporated as a potent antimicrobial ingredient in a range of commercial products. However, its mode of action is largely uncharacterised. To address this, the cellular response to Compound X exposure was studied in two Gram-negative species, *Escherichia coli* and *Serratia marcescens*, and one Gram-positive, *Staphylococcus aureus,* using inductively-coupled plasma mass spectrometry. In the two Gram-negatives, Compound X caused a decrease in cellular iron levels, combined with an increase in cellular manganese, consistent with a cellular response to compensate for the iron starvation. Contrastingly, Compound X induced an increase in cellular iron, manganese and zinc levels in *S. aureus*. Transcriptional analysis of metal-response genes, proteomics and Compound X-metal combination checkerboard assays revealed that iron availability is reduced in all three species. I propose a model in which Compound X sequesters iron at the cell surface of Gram-positive cells, which is responsible for its antimicrobial efficacy. In Gram-negative species, differences in the envelope structure, notably the lipopolysaccharide-rich outer membrane, prevents Compound X association meaning that iron is primarily sequestered from the extracellular environment.

A229

The effects of copper on *Staphylococcus aureus* USA300 fitness and how this shapes interactions with the host

Leighton Blake-Greaney[1], Ayat Ali[1], Joan Geoghegan[2], Kim Hardie[3], Rian Griffiths[3], Julie Morrissey[1]

[1]University of Leicester, Leicester, United Kingdom. [2]University of Birmingham, Birmingham, United Kingdom. [3]University of Nottingham, Nottingham, United Kingdom

Abstract

Copper is utilized by the host innate immune system as an antimicrobial against bacterial pathogens. Many bacteria have evolved copper tolerance systems to prevent the intracellular accumulation of copper and thus reduce its toxicity. Our group focuses on *Staphylococcus aureus* clone USA300 and how this is affected by copper.

Common to all *Staphylococci* is the *copAZ* operon encoding a copper-efflux pump (CopA) and a copper-binding metallochaperone (CopZ) shown to bind copper and deliver it to CopA for export). USA300 has acquired an additional operon called *copXL* encoding a copper-efflux pump (CopX) and a copper-binding surface lipoprotein (CopL), conferring copper hyper-resistance. Previously, our group and others have shown these core and additional copper tolerance genes are important for USA300 survival in macrophages in addition to murine respiratory and skin models of infection.

Here we have generated single gene mutants for each of these key genes and investigated how they respond to copper on both transcriptomic and phenotypic levels. Transcriptomics suggest that copper is used as a signal, driving the differential expression of >100 genes, including many associated with metabolism, cell wall architecture and virulence. Here we explore the effects of copper on USA300 fitness in greater detail and how this shapes interactions with the host.

A230

Exploring Lanthanide Biochemistry: Methanol Metabolism in Archaea

Kyle Lowry, Aileen Lynch, Chris Allen

Queen's University Belfast, Belfast, United Kingdom

Abstract

Lanthanide-binding alcohol dehydrogenases are a class of enzymes with the ability to catalyse alcohol oxidation using rare earth elements as co-factors. The XoxF family of lanthanide-binding methanol dehydrogenase enzymes serve as a model for understanding how lanthanides can support biochemical processes in enzymes that typically use calcium as a co-factor. This study aims to expand our knowledge of lanthanide biochemistry by identifying potential lanthanide-binding alcohol dehydrogenases in archaea. Given the unique adaptations of archaea to extreme environments, discovering lanthanide-dependent enzymes in this domain could significantly broaden our understanding of biochemical diversity and evolutionary adaptation. By focusing on alcohol dehydrogenases similar to XoxF in archaea, this research sheds light on the potential role of lanthanides in archaeal metabolism—a previously unexplored area.

The study used computational tools to predict the structure and function of these enzymes *in silico*. AlphaFold2 enabled structural prediction, while DeepAlign was used for 3D structural alignment, and PyMOL facilitated detailed protein visualization. *In vitro* experiments were conducted by growing several haloarchaeal species in a defined medium with lanthanides and either methanol or ethanol as the sole carbon source.

Structural analysis of these enzymes *in silico* has supported the prediction that these enzymes are lanthanide-dependent alcohol dehydrogenases. The growth of haloarchaeal species on methanol and ethanol supports the hypothesis that these organisms possess lanthanide-dependent alcohol dehydrogenases. This expands the biochemical role of lanthanides to the domain Archaea and also suggests the ability of archaea in extreme methanol-rich environments to catabolise methanol as a carbon source.

A231

Genomic characterization and identification of six clinical wound isolates

Taiwo Akinyele

NOTTINGHAM, NOTTINGHAM, United Kingdom

Abstract

Background: Infectious diseases continue to pose significant challenges to global health and the economy. This study examines the application of whole genome sequencing data to six clinical isolates to characterize and identify their antimicrobial resistance profile and determine their virulence factor and prophages.

Method: Clinical strains were collected from Nottingham University Hospitals Trust, UK. The genomes were sequenced via Illumina sequencing. The genomes' unique genomic properties were identified using the Comprehensive Antibiotic Resistance Database, virulence factor database, and Phage Search Tool Enhanced Release.

Results: *P*. *aeruginosa* strains (SMC 067 and SMC 068) showed the highest AMR attributes, followed by the Escherichia *coli* (SMC 064) strain. *S*. *aureus* strains (SMC063 and SMC066) exhibited the lowest AMR profile distribution. The virulence factor database revealed that the genomes of the bacterial isolates harbored different frequencies of virulence markers associated with multiple virulence factor classes, including adherence, enzyme, immune evasion, iron uptake, regulation, secretion system, and toxin secretion. Phaster revealed that the isolates are likely prophage-containing
isolates.

Discussion: Several research studies have revealed that several antibiotic-resistance genes, phages, and virulence factors in bacterial isolates contribute to their pathogenicity. *S*. *aureus* is an adaptable pathogen harboring various genetic elements related to virulence and niche adaptation, resulting in many infections in people and animals. Antibiotic efflux, antibiotic target alteration, antibiotic target replacement, and antibiotic inactivation were the primary mechanisms linked to the *S*. *aureus* antimicrobial-resistance trait.

A232

Characterisation of novel sugar metabolism pathway in the human bacterial pathogen Streptococcus pyogenes

Joshua Horton[1,2], Sona Jabang[1], Rebecca Kirtland[1], Henna Khalid[1], Claire Turner[1]

[1]University of Sheffield, Sheffield, United Kingdom. [2]Quadram Institute, Norwich, United Kingdom

Abstract

The human pathogen, *Streptococcus pyogenes* (also know as group A Streptococcus or Strep A) predominantly causes infections on the skin or in the throat, but it can also cause more severe invasive disease in different parts of the body. The skin and the throat are very different environments and as such *S. pyogenes* must be adaptable to survive under different conditions and use different sources of metabolites.

Our recent study, comparing skin-infection causing *S. pyogenes* isolates from the UK with those from The Gambia, West Africa identified a locus encoding for an unknown potential alternative sugar-metabolism pathway. This locus was much more common in isolates from The Gambia compared to those from the UK. BLAST analysis predicted the presence of a beta-glucuronidase (*uidA*). We therefore hypothesised that it would allow the bacterium to utilize glucuronic acid as a carbon source.

Molecular analysis of the locus confirmed that indeed *uidA* was a beta-glucuronidase but that the locus included a gene encoding for a second beta-glucuronidase (*nag3*). The locus also included a gene encoding for a protein responsible for sugar import (*yag*G) as well as a negative regulator, *fadR*. Expression of the pathway varied between isolates but allows for the utilisation of different carbon sources compared to those who do not carry the genes for this pathway. This may drive tissue-trophism and potentiate skin infections.

A233

Mycoviruses in Aspergillus fumigatus and their effect on host susceptibility to anti-fungal agents

Wynn Jenkins[1], Sophie Appleyard[1], Anna Szabo[1], Robert Coutts[2], Ioly Kotta-Loizou[1]

[1]Imperial College London, London, United Kingdom. [2]University of Hertfordshire, Hatfield, United Kingdom

Abstract

Aspergillus fumigatus is a fungus of clinical significance, especially in cases involving immunosuppression, like organ-transplant recipients and oncological patients. Treatment options are limited, and thus investigation into alternatives is paramount. Thus far, our group have explored how viral infection in fungi infection influences host sensitivity to anti-fungal agents, quantified using XTT assays. For this investigation, we used *A. fumigatus* polymycovirus AfuPmV-1 infected, cured, and reinfected strains. We identified that a pore-forming antimicrobial peptide termed 'peptide 1' impacts AfuPmV-1 infected *A. fumigatus* strains more than uninfected strains. However, we found no statistical significance between mycoviral infection and reaction to antifungal azoles (voriconazole and itraconazole), as well as showing that synthetic sputum medium (SSPM) more closely replicates human lung environment than Roswell Park Memorial Institute (RPMI) medium. We showed that even when using SSPM instead of RPMI, AfuPmV-1 fails to alter voriconazole susceptibility, and that acetyl CoA synthetase inhibitor (AR12) has inconsistent impacts on metabolic activity, depending on mycoviral strain. The aim going forward with this project is to test for differences in effect of intracellular anti-fungals (like Olorofim, VL-2397, T-2307 and MGCD2), as well as combinations (like Voriconazole and AR-12), on AfuPmV infected and uninfected strains, quantifying metabolism using XTT assays.

Hence through expanding the understanding of mycoviral influence on anti-fungal drug efficacy, we can optimise existing treatments for aspergillosis, and potentially generate new clinical applications for drugs not previously used for these specific anti-fungal properties.

A234

The impact of particulate matter (PM) air pollution on biofilm formation and proteomic profiles of commensal bacteria of the skin and respiratory tract

Lillie Purser, Angelica Aleksandar, Julie Morrissey

University of Leicester, Leicester, United Kingdom

Abstract

The role of the microbiome in health and disease states has now been well documented, and while there has been much research into the impacts of diet and health interventions on the microbiome, other external influences have been overlooked. Our research showed for the first time that black carbon (BC), a major component of particulate matter (PM) air pollution, changes the behaviour of a variety of opportunistic pathogens that are resident within the respiratory microbiome, including *Streptococcus pneumoniae* and *Staphylococcus aureus*. The ability of BC to induce changes in biofilm formation and virulence factor expression demonstrates that air pollution does not only directly harm the host, but also induces changes in pathogenic bacteria, and therefore has the potential to impact the bacteria within the microbiome as a whole.

Recent work from our group has focussed on the impact that PM exposure has on the commensal bacteria within the lung and skin microbial communities, with a focus on *Staphylococcus epidermidis*, an important member of the skin microbiome that is known to be beneficial in atopic dermatitis (AD), but is also known to cause serious clinical complications in patients with indwelling medical devices. The effects of BC and brake dust, another major component of PM, on the biofilm formation and proteome of *S. epidermidis*, as well as respiratory commensals *Prevotella melaninogenica* and *Rothia muciliganosa* will be discussed.

A235

Quantification comparisons of different genomic targets through bacterial growth stages

Daniel Evans[1], Graham Stewart[2], Denise O'sullivan[1]

[1]LGC group, Teddington, United Kingdom. [2]University of Surrey, Guildford, United Kingdom

Abstract

Quantitative molecular tools such as PCR are increasingly used to count the abundance of bacterial cells within a variety of materials, such as environmental analysis or clinical samples. However, as PCR quantifies targeted regions of genomic DNA, these measurements may be susceptible to bias due to target sequence position because of bacterial DNA replication. Digital PCR was used to quantify three different genomic targets including the origin of replication (*oriC*) and terminus (*ter*) of *Escherichia coli* during culture, with results showing a 2.5-fold difference in quantification between oriC and ter while cultures were in logarithmic growth phase. Additionally, commercial control materials were assessed to demonstrate how this differential quantification may impact on real samples and standard materials.

This project presents important information on understanding assay bias as a result of genomic position, and how this should be considered for both manufacturers of control materials and end users. Additionally, improving the understanding of the relationship between genomic sequence abundance through bacterial growth and the methodology to accurately assess this via multiplex quantitative assays may have applications to improve the information which can be obtained via molecular testing environmental and clinical samples.

A236

Structural Characterisation of Lysogenic Phage from the Liverpool Epidemic Strain of Pseudomonas aeruginosa

Andrew martin[1], Enrique Gonzáles-Tortuero[2,3], Revathy Krishnamurthi[4], Reem Almeran[3], Ian Goodhead[1], Jo Fothergill[3], Heather Allison[3], Chloë James[1]

[1]Salford University, Salford, United Kingdom. [2]Northumbria University, Newcastle, United Kingdom. [3]Liverpool University, Liverpool, United Kingdom. [4]Quadram Institute, Norwich, United Kingdom

Abstract

The Liverpool Epidemic Strain (LES) of Pseudomonas aeruginosa is a key opportunistic pathogen and major cause of respiratory morbidity and mortality in people with cystic fibrosis (CF). A set of active prophages (LES ϕ2-6) have been associated with fitness advantages of LES.

This study prepared high titre, purified suspensions of LES phages ϕ2, ϕ3 and ϕ4 through infection of the well-characterised susceptible host strain PAO1 and carried out structural analysis. Transmission electron microscopy (TEM) confirmed that each phage exhibited a Siphoviridae morphology with icosahedral capsid heads (50-60 nm diameter) and long flexible tails (~200 nm long). Tail fibre structures were clearly visible at the end of LESϕ4 tails. Following SDS-PAGE separation of purified phage suspensions, proteomic analysis detected 11, 8 and 9 structural proteins for LES phage ϕ2, ϕ3 and ϕ4, respectively, which is more than those identified using genome annotation tools. Amino acid sequencing of excised protein bands (ranging in size from 6.8 to 89.3 kDa) confirmed the identity of suspected structural proteins. Further structure-based analysis using AlphaFold, HHPred and the UniProt database predicated alternative putative functions for the unidentified proteins. Mutational work is ongoing to determine the actual function of a 21.8 kDa protein of previously unknown function, evidence suggests it is potentially a CRISPR CAS protein or a phage head-to-tail adaptor protein.

Overall, this study used a combination of genomic, proteomic, TEM, and functional analyses to improve our understanding of LES phage biology.

A237

Development of single-cell mass spectrometry to understand metabolic differences in live *Mycobacterium bovis* BCG infected macrophages and their uninfected bystanders.

Abigail Cook ORCID iD, Dany Beste ORCID iD, Melanie Bailey ORCID iD

University of Surrey, Guildford, United Kingdom

Abstract

Mycobacterium tuberculosis is once again the leading cause of infectious death globally. Metabolic crosstalk between *Mycobacterium tuberculosis* and its human host cell determines the outcome of an infection, yet we still have a poor understanding of this metabolic interaction. Studies at the population level have demonstrated that amino acids have a central role in this process. Amino acids are involved in host cell and bacterial defensive mechanisms and are also critical nitrogen sources. Our work aimed to explore the role of amino acids at the single-cell level to test the hypothesis that amino acids drive bystander effects in tuberculosis infection.

We utilised spatially resolved capillary sampling to selectively extract single mycobacterial infected and bystander uninfected human macrophages and applied mass spectrometry methods to measure amino acids in these cells. Using the Yokogawa Single Cellome™, we successfully sampled single, live *Mycobacterium bovis* BCG infected macrophages, their uninfected bystanders and uninfected control cells into nanocapillaries under confocal microscope observation. Along with amino acids, we were able to detect a number of other metabolites and identify those that caused most variance in each cell type. This analysis allowed us to show that bystander uninfected macrophages had a different metabolic profiles to both infected and control uninfected macrophages, suggesting cellular communication protected these cells from being infected. Understanding this metabolic phenomenon will allow us to develop host directed therapies as adjuvants to antibiotics in the future.

A238

Clade-Specific Phenotypes and Genetic Basis of Salmonella Enteritidis in Sub-Saharan Africa

Ahmed Hazazi[1,2], Jay Hinton[1], Blanca Perez Sepulveda[1], Modupeh Bretts[1], Xiaojun Zhu[1], Aisling Brady[1], Lijuan luo[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Jazan University, Jazan, Saudi Arabia

Abstract

Invasive non-typhoidal *Salmonella* (iNTS), especially S. Typhimurium and *S.* Enteritidis, significantly impact public health in Sub-Saharan Africa causing bloodstream infections (BSI), especially among immunocompromised individuals.

This study investigate clade-specific phenotypes of two emerging *S.* Enteritidis lineages identified in Africa, the Central/East African Clade (CEAC) and West African Clade (WAC) and also to elucidate the genetic basis underlying clade-specific carbon source utilisation.

Using 152 *S.* Enteritidis isolates mostly isolated from human blood between 1998-2021, we identified distinctive carbon source utilisation patterns in each clade. Both clades lacked the ability to metabolise melibiose and galactitol independently, though CEAC isolates utilised galactarate, and WAC isolates uniquely metabolised 1,2-propanediol. Biofilm formation also differed; WAC isolates produced the RDAR biofilm morphology, a resistant biofilm structure thought to play an essential role in *Salmonella* transmission, while CEAC isolates displayed smooth, non-biofilm-forming phenotypes, suggesting biofilm-formation transcription could be shut-off due to a SNP mutation.

WAC and CEAC isolates exhibited multidrug resistance, particularly to first and second-line antibiotics, yet oxidative stress resilience was universal among all isolates, underscoring a non-clade-specific phenotype.

Functional analyses of metabolic genes (melR, pocR, gatC, gatZ, gatR, and *CRP*) revealed a *melR* SNP in strain D7795, affecting melibiose metabolism, and a *pocR* frameshift mutation inhibiting propanediol utilization—traits reversible by complementation with a functional gene. Knockouts in the galactitol operon genes confirmed their role in growth deficiency, highlighting gatR's regulatory role.

These insights into clade-specific adaptations contribute to strategies for addressing the spread of iNTS in Africa.

A240

The role of copper in modulating bacterial interactions within the oral cavity.

Safa Chogule

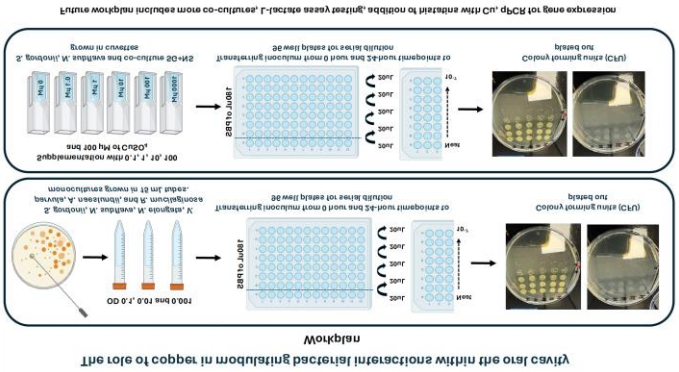
Durham University, Durham, United Kingdom

Abstract

The oral cavity is a diverse microbiome consisting of over 700 bacterial species which is regulated by a balance of microbial interactions, salivary components, and environmental factors which prevent dysbiosis. Metal ions are vital components of the host immune system. Copper plays a dual role as an essential bacterial micronutrient at low concentrations and potent antibacterial toxins at higher concentrations. In the oral cavity, Cu ions have been implicated in biofilm formation, stress response, and bacterial pathogenesis. However, copper metabolism in oral bacteria remains uncharacterized.

This project aims to elucidate the dual pro- and anti-bacterial role of copper against the oral microbiome. Monoculture assays were conducted to establish growth for several oral bacteria, namely *Streptococcus gordonii*, *Neisseria subflava*, *Neisseria elongata*, *Veillonella parvula*, *Actinomyces naeslundii*, and *Rothia mucilaginosa*. *S. gordonii* and *N. subflava* were selected for co-culture experiments based on their different copper utilisations. When supplemented with Cu (0.1 µM – 100 µM), no significant impact is seen with *S. gordonii*, however, an increase in CFU counts is seen with *N. subflava.* When grown in a co-culture, the two species displayed an antagonistic interaction, with *S. gordonii* outcompeting *N. subflava.* Supplementation with Cu did not rescue *N. subflava* in co-culture experiments. These findings highlight the potential of copper to modulate interspecies dynamics within the oral microbiome.

Studies that examine the biochemical mechanisms that underpin the above observations are ongoing. In the future, the effects of copper-binding salivary proteins and peptides will be explored.



A241

Effects of simulated microgravity on the Candida albicans/Staphylococcus aureus dual species microbial community.

John Munnoch[1], Rebecca Craig[1], Umme Nur Habiba[2], Brian Patton[1], Gail McConnell[1], Paul Hoskisson[1], Jessica Lee[3], Katherine Baxter[4,1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]California State University, Long Beach, USA. [3]NASA Ames Research Center, Moffet Field, USA. [4]University of Glasgow, Glasgow, United Kingdom

Abstract

Spaceflight conditions cause detrimental changes to the immune, cardiovascular and musculoskeletal systems. These changes are reminiscent of health conditions faced by immunocompromised and elderly populations on Earth, suggesting astronauts may be at risk to similar health issues faced by these patient populations, including opportunistic infections caused by components of the skin microbiome.

Two common skin microorganisms found together in these infections are *Candida albicans* and *Staphylococcus aureus,* which cause hard to treat disease resulting in poorer patient outcome than infection with either species alone. To date, there are no studies on the effects of microgravity on the behaviour and virulence of the *C. albicans/S. aureus* dual species microbial community, leaving the potential risk to crew from this common synergistic pairing unknown.

Using the Cell Spinpod rotation suspension culture system to create simulated microgravity conditions, we are comparing simulated microgravity grown multispecies cultures of *C. albicans*/*S. aureus* to 1g controls. Our preliminary data indicate simulated microgravity impacts several *C. albicans/S. aureus* virulence factors including biofilm formation and variations in hyphae. Population ratios between simulated microgravity and 1g are also observed, indicating shifts in community behaviour.

By investigating the impact of microgravity on skin microorganisms commonly associated with infections in health-compromised terrestrial populations, we can further our understanding of the microbiome as a reservoir of infection during spaceflight, and develop countermeasures which can be applied in both space and Earth-based medicine.

Session : The Role of Microbiomes in Humans, Animals, and Ecosystems

A245

The Effects of Kratom on Gut Microbiome Alterations: Insights from a Rat Model Study

Phornphan Sornchuer[1], Nattaya Thongsepee[1], Sumet Amonyingcharoen[2], Pholasit Chamod[1]

[1]Department of Preclinical Science, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand. [2]Medical Life Sciences Institute, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand

Abstract

Kratom (*Mitragyna speciosa*), a plant native to Southeast Asia, is recognized for its diverse pharmacological properties, including anti-inflammatory and antibacterial effects. Despite its rising recognition and potential therapeutic uses, the effects of kratom on gut microbiota in rat models have yet to be thoroughly investigated. This study aims to evaluate the effects of kratom extract on gut microbiota alterations in a rat model, employing metagenomic sequencing of 16S rRNA gene. Male Wistar rats were divided into two groups (*n* = 6 per group) and maintained on commercial pellet food and reverse osmosis water. Kratom extract was administered via oral gavage at a dose of 300 mg/kg body weight daily for 28 days. Fresh fecal samples were collected for gut microbiota analysis, and metagenomic sequencing of the prokaryotic 16S rRNA gene was performed. Eight classes of bacteria were identified in the feces of the rats. Notably, *Actinobacteria* significantly decreased in kratom-treated rats. Among the 18 bacterial orders analyzed, only *Corynebacteriales* showed a significant reduction. Additionally, of the 30 bacterial families examined, *Corynebacteriaceae* significantly decreased, while *Erysipelatoclostridiaceae* significantly increased following kratom administration. The results indicate that kratom extract can alter gut microbiota in experimental animals. Alteration in gut microbiota has been linked to various diseases, suggesting that manipulating gut microbiota through plant-based interventions may provide therapeutic potential. However, certain alterations in gut microbiota may also be implicated in the pathogenesis of specific diseases. Therefore, further research is warranted to elucidate the mechanisms underlying these microbiota changes and their health implications.

A247

What happens in the gut doesn't stay in the gut

Lesley Hoyles

Nottingham Trent University, Nottingham, United Kingdom

Abstract

The gut microbiota is essential to human health and its modulation is implicated in a range of conditions, including obesity, type II diabetes, neurodegenerative diseases and cancers. Gut bacteria produce metabolites that are taken up from the gut and enter the circulation unchanged, or which are modified by human phase I/II metabolism to produce a range of host-microbiota co-metabolites. These microbiota-associated metabolites (MAMs) interact with organs throughout the body. It is becoming clear from mechanistic work around cardiometabolic diseases, cancer therapeutics, chronic kidney disease and brain function that MAMs can 1) be exploited to improve host metabolic and chemotherapeutic responses, and 2) influence disease phenotypes. This offered talk will summarize our current knowledge in the nascent, but important, field of pharmacomicrobiomics as applied to improving our understanding of host-MAM interactions.

A248

High-throughput screening of bio-insecticides against mosquito vectors

Jie Wang, Vandana Prasad, Kona Prasad, George Dimopoulos

Johns Hopkins University, Maryland, USA

Abstract

The increasing use of chemical insecticides always leads to resistance. Bio-insecticides, as eco-friendly, cost-effective alternatives, are not likely to encounter resistance. In this study, we extracted and grew bacteria from 48 different soil samples collected in Puerto Rico, obtaining 510 different colonies and performing high throughput larval bioassays. We have identified 15 colonies that exhibit mortality rates ranging from 80% to 100% against both mosquito larvae and adults after 24 hours of incubation (Aedes aegypti Liverpool, Culex quinquefasciatus, and Anopheles stephensi). We further conducted experiments to determine the growth conditions for these 15 selected bacteria to boost their optimizing toxicity against mosquito vectors. So far, we found that for *Serratia.marcescens* when they are grown on YPD medium at 28 degrees Celsius aerobically for 1 day followed by anaerobically for 3/5/7 days, they show the highest mortality rate towards Aedes Liverpool larvae. *Citrobacter.freundii* grown aerobically for one day followed by anaerobically for 5 days on LB medium at 28 degrees show the highest killing ability towards Aedes Liverpool larvae. Finally, for Acinetobacter, the optimal conditions would be on LB medium at 28 degrees Celsius aerobically grown for 1 day followed by anaerobically grown for 3,5 or 7 days. In the future, we will continue experimenting with the remaining selected bacteria to develop a formula suitable for large-scale industrial production.

A249

From soil to guts: how the microbiome discourse integrates agriculture and healthcare

Shana Hepping ORCID iD

Leiden University, The Hague, Netherlands

Abstract

The integration of agri-food and healthcare presents a promising pathway to address the complex challenges of human and planetary health. This study explores the emerging discourse around the microbiome as a transformative connector between the food system and healthcare. Using discourse analysis and the leverage points perspective, we analyze narratives of stakeholders across the Dutch food chain and healthcare sectors, including farmers, dieticians, researchers, and project managers, gathered through semi-structured interviews. The findings highlight how the microbiome is framed not only as a biological entity but also as a symbol of systemic interconnectivity, guiding a shift towards more holistic approaches to food and health. By categorizing these narratives based on microbiome potential, system characteristics, and barriers to transformation, we identify leverage points that can support a paradigm shift in both domains. Our analysis suggests that the microbiome discussion is not just a passing scientific trend but a powerful force for rethinking the relationship between agriculture and healthcare. These insights have significant broader implications for promoting collaborative initiatives that bridge these two sectors, promoting a One Health perspective that encompasses environmental sustainability, sustainable and stable food production, and public health.

A250

The Role of Flagellar Motility in Bacterial-Fungal Endosymbiosis

Ariel Heinrich, David Baltrus ORCID iD

University of Arizona, Tucson, USA

Abstract

Characterizing inter-kingdom microbial interactions has proven instrumental in understanding important ecological relationships both in natural environments and in agricultural settings. To this end, multiple types of bacteria have been shown to interact intimately with fungi, invading their hyphae to form an endosymbiosis. However, little is known about the mechanisms enabling such interactions. A species of cypress-associated endophytic fungus, an ascomycete in the genus *Pestalotiopsis*, was found to harbor the facultatively endosymbiotic bacterium *Luteibacter mycovicinis.* Both bacteria and fungi are culturable in isolation and can be reassociated together *in-vitro*. We hypothesize that flagellar motility might play a critical role in this establishment process. Using targeted mutagenesis, we have created a strain with deletions in the flagellar genes FlgB and FlgC of endosymbiotic *Luteibacter*. Reassociation assays were then performed using these deletion strains by co-culturing bacteria and fungi in minimal media, treating the culture with gentamicin to eliminate extrahyphal bacteria, and transferring the reassociated organisms onto water agar. To quantify endohyphal bacteria, cores were sampled from the water agar plate, and a dilution series was plated on nutrient agar containing an antifungal. We report that strains with impairments in motility show significantly less reassociation than wild-type controls, demonstrating that flagellar motility is necessary for successful establishment of endosymbiosis. Further investigation of the motility-associated genes in endosymbiotic *Luteibacter* strains may allow for increased understanding of the mechanism of entry for endohyphal symbionts such as these, as well as the impact that these interactions have on the host organisms and the larger ecosystem.

A251

The Hand Microbiome and Antimicrobial Soaps

Sara Henderson ORCID iD[1], Megan Stenton[2]

[1]Centre for Skin Sciences, School of Chemistry and Biology, University of Bradford, Bradford, United Kingdom. [2]School of Chemistry and Biology, University of Bradford, Bradford, United Kingdom

Abstract

Hand eczema is increased in persons with high frequency hand washing. During this condition the healthy diverse microbiome shifts towards a microbiome dominated by *Staphylococcus aureus*. It is unclear if antimicrobial soaps and hand washing are directly influencing this shift. To investigate if different groups of persons have altered sensitivity to antimicrobial soaps the microbiome of persons with or without a history of childhood eczema but currently not suffering from any inflammatory diseases were isolated on Columbia blood agar and mannitol salt agar. The group with a history of eczema produced lower diversity of colony morphologies on the initial isolation plates. Identification to the genus level was achieved by amplification and sequencing of the 16S rRNA. This indicated that Staphylococcus was isolated most frequently on Columbia Blood agar from both healthy and those with a history of eczema. Interestingly, no *S. aureus* was isolated from any individual within this study. To investigate if any differences in soap sensitivity exist between the different groups sensitivity testing was performed via disk diffusion MIC to 21 commercially available liquid hand soaps. All strains tested demonstrated sensitivity to the common topical antibiotics fusidic acid and neomycin. Sensitivity to the soaps varied from no inhibition to zones of inhibition greater than 30 mm, with all soaps exhibiting antimicrobial ability to at least one tested strain. Ongoing work will compare the patterns of inhibition and if any of the strains present with antimicrobial activity against *S. aureus* or *Pseudomonas aeruginosa*.

A252

Role of Immunostimulatory Deoxycytidylate-Phosphate-Deoxyguanylate (CpG) Motifs in Oral Bacteria Associated with Oral Diseases

Pisit Charoenwongwatthana ORCID iD[1,2], Oslovenya Caroline[1,3], Chien-Yi Chang ORCID iD[1], Jamie Coulter ORCID iD[1], Halah Ahmed ORCID iD[1]

[1]Newcastle University, Newcastle, United Kingdom. [2]Mahidol University, Bangkok, Thailand. [3]Universitas Indonesia, Jakarta, Indonesia

Abstract

Aim: This study investigates the immunostimulatory CpG motifs in the genomes of oral bacteria associated with endodontic disease, periodontal disease, and dental caries to elucidate their pathogenic influence on the host immune responses.

Methodology: Fifty oral bacterial genomes associated with endodontic disease, periodontal disease, and dental caries, were extracted from the expanded Human Oral Microbiome Database (eHOMD), with each entry accompanied by its respective National Center for Biotechnology Information (NCBI) GenBank Accession ID. *In silico* analyses were conducted to determine the GC% content and the frequency of CpG motifs within each genome. Correlation analysis was performed to establish the relationship between GC% content, CpG motifs frequency, and genome size. Subsequent normalisation of sequences was implemented to enable unbiased comparison of frequency counts among oral bacteria.

Results: Sixty percent of oral bacteria exhibited medium GC% content (*Mdn* = 44). No significant differences in GC% content were found among these bacteria ($p = 0.66$). Correlation analysis indicated a positive relationship between GC% content and CpG motifs frequency, as well as genome size and CpG motifs frequency. A higher-than-average GACGTT frequency (optimal immunostimulatory sequences for mice and rabbits, 9/14) and GTCGTT frequency (optimal immunostimulatory sequences for humans, 7/14) was observed in the majority of core endodontic microbiota.

Conclusion: CpG motifs in oral bacteria implicated in endodontic disease, periodontal disease, and dental caries might play a role in diseases progression through the modulation of host immune responses. Treatment strategies targeting bacterial CpG motifs could represent a promising avenue for novel therapeutic interventions.

A253

MICROCOSM - The gut MICRObiome and COgnition in SubMariners

Miriam F Hares ORCID iD, David Inglis, Emily M Wilson ORCID iD, Andrew P Bagshaw ORCID iD, Jonathan C Catling ORCID iD, Willem Van Schaik ORCID iD, Matthew K O'Shea ORCID iD

University of Birmingham, Birmingham, United Kingdom

Abstract

The human gut microbiome is a complex ecosystem that shifts in response to numerous environmental and genetic factors. Changes in composition and diversity can affect the functional output of the gut microbiota leading to biochemical changes in the brain via the Gut-Brain Axis (GBA) that can shape cognition, mental health and sleep quality. Submariners must sustain high levels of alertness throughout extended deployments to operate their submarine safely and to take effective action during emergencies or hostile engagement. This unique operating environment poses a significant challenge to the maintenance of a submariner's optimal performance.

With the purpose of defining candidates for novel interventions that may mitigate this challenge, a longitudinal prospective cohort study was conducted to determine the associations between the compositional and functional aspects of the faecal microbiome and the outcomes of psychological testing in submariners. Faecal samples were collected prior to, during and post-deployment at 4 weekly intervals. At each collection, psychological tests were completed by participants to quantify cognitive performance, and sleep and activity data was monitored continuously. Samples underwent DNA extraction, shotgun metagenomic sequencing and analysis. A submarine crew serves as a unique study population that is essentially quarantined for extensive periods, which negates many confounders that afflict conventional study populations. We hypothesised that the microbiomes of submariners would conform over time to a specific microbial diversity, composition, and functionality from which predictors of mental health, cognitive performance and sleep quality would be established.

A255

Unmasking the mechanisms behind the Immunomodulatory effects of Bifidobacterium breve UCC2003

Charlotte Jones[1], Ian O'Neill[2], Sree Gowrinadh Javvadi[2], Douwe van Sinderen[3], Lindsay Hall[1,2]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Quadram Institute Bioscience, Norwich, United Kingdom. [3]University College Cork, Cork, Ireland

Abstract

Establishing a healthy gut microbiota is crucial for overall health, with *Bifidobacterium* species and strains widely recognised for their beneficial effects during early life and widely used as probiotics. *Bifidobacterium breve* UCC2003, originally isolated from the stool of a breast fed infant, has demonstrated protective effects against bacterial infections and improve gut barrier function in neonatal mice. However, the mechanisms driving these health benefits remain largely unknown.

In this study, we applied a genome-wide random mutagenesis approach using a *B breve* UCC2003 Tn5 insertion library to investigate gene-specific influences on inflammatory responses. Notably, specific mutant strains showed a marked ability to modulate inflammation in a human macrophage-like cell line, by altering nuclear factor (NF)-κB activation, a key transcription factor involved in inflammatory responses. Conditioned media from these mutant strains demonstrated dose-dependent effects, either increasing or decreasing NF-κB activation, suggesting that the secreted metabolite and protein pool plays a central role in mediating the anti-inflammatory/pro-inflammatory effects observed.

Given that NF-κB is often overactivated in macrophages isolated from Inflammatory Bowel Disease (IBD) patients, leading to elevated inflammatory cytokine levels, our ongoing work aims to determine whether these *B. breve* mutants and their secreted metabolites/proteins exhibit similar modulatory effects on IBD patient-derived macrophages. By pinpointing the genes and purifying the molecules responsible for anti-inflammatory effects, this research holds potential for developing an alternate therapy using *B. breve* UCC2003 to mitigate chronic inflammation in IBD patients.

A256

Unlocking the Potential of the Human Ocular Microbiome: New Sources in the Battle Against Antibiotic-Resistant S. aureus?

Holly Richardson, Mal Horsburgh, Kier Nicholas-Haizelden

University of Liverpool, Liverpool, United Kingdom

Abstract

Staphylococcus aureus (*S. aureus*) has emerged as a major cause of bacterial infection worldwide, posing a significant threat to public health. The rise of antimicrobial drug resistance intensifies the challenges of treating *S. aureus* infection, necessitating innovative sources of novel antimicrobials. While the human microbiome, particularly the human nasal microbiome, shows promise as a novel source, a substantial knowledge gap exists regarding the human ocular microbiome as another potential source. Therefore, the aim of this project was to explore the possibility that ocular microbiome isolates of *Staphylococcus epidermidis* (*S. epidermidis*), are a source of novel antimicrobials.

To identify whether ocular *S. epidermidis* could serve as a source of novel antimicrobials for *S. aureus*, a series of deferred growth inhibition assays were carried out to identify *S. epidermidis* bacteria that had inhibitory capability against *S. aureus*. The genomic DNA sequence of the inhibitory *S. epidermidis* strains was then examined for a genome-level analysis of the bacterial gene clusters responsible for biosynthesis, to identify novel antimicrobial compounds.

Among the ocular strains studied, *S. epidermidis* 039331N1 was found to inhibit *S. aureus* and produced an undescribed antimicrobial compound. This raises the possibility that the human ocular microbiome serves as a source for novel antimicrobials, which could prove useful in addressing the challenges posed by antibiotic-resistant *S. aureus*.

A257

The effects of environmental variation on the stickleback fish skin microbiome.

Francis Gyapong ORCID iD[1,2], Tamar Guetta-Baranes[1], Andrew MacColl[1], Fiona Whelan[2]

[1]Universityof Nottingham, Nottingham, United Kingdom. [2]University of Manchester, Manchester, United Kingdom

Abstract

While the rise in antimicrobial resistance has been associated with human- and livestock-dominated environments, its wider environmental occurrence is poorly understood. Emerging evidence suggests that resistance to metals and antibiotics is often co-selected in bacterial genomes and directly correlates with environmental metal levels. Here, we use culture-dependent and -independent methods to study the skin microbiome of the three-spined stickleback, a model organism native to fresh and oceanic northern waters. We aim to determine whether varying metal concentrations on the island of North Uist, Scotland, encourage the spread of resistance genes in the fish skin microbiota. Culture-dependent methods involve plating the skin microbiome sample on an array of agars to grow the total diversity of the microbiome, whereas culture-independent methods describe the use of 16S rRNA gene sequencing of samples collected by swapping the skin of individual stickleback fish. The stickleback skin microbiota analysis from 17 freshwater lochs (lakes) shows variation in the relative abundance of multiple bacterial genera. Most of the abundant genera – including *Janthinobacterium*, *Pseudomonas*, *Chryseobacterium*, and *Acinetobacter* – have been reported as members of the skin microbiota of other fish species, suggesting that these taxa are commonly associated with different types of fish species. Further, our culture-dependent approach revealed similar relative abundances of common genera identified in our culture-independent samples but captured a greater abundance of genera – such as *Arthrobacter* and *Serratia* – which were low abundance in the swabs. Future investigations will determine if the differences in the microbiota we observed between lakes correlate with differences in environmental variation.

A258

The plot thickens: The impact of mucus and the microbiome on *Staphylococcus aureus* nasal colonisation.

Megan Smith-Cerdán ORCID iD, Justine Rudkin ORCID iD

University of Glasgow, Glasgow, United Kingdom

Abstract

Staphylococcus aureus leads a double life- as both a leading cause of healthcare-associated mortality and a natural human commensal. 30-50% of adults are carriers at some point, and colonisation is a big risk factor for infection development. The primary niche for *S. aureus* colonisation is the nose, and epithelial cell attachment is key in this host-bacteria interaction. These cells are covered by a protective mucus layer, serving as a potential nutrient source for the nasal microbiome. Interestingly, *S. aureus* lacks common virulence factors, such as flagella and mucolytic enzymes, that would typically aid in mucus penetration and utilization, raising important questions about how *S. aureus* establishes and thrives in this mucus-rich environment.

Preliminary data shows that *S. aureus* grows poorly when mucin is the sole carbon source, unlike other nasal species with the capacity to hydrolyse mucin. Mutualistic mucin breakdown and cross-feeding is well-known in the gut, but not in the nasal microbiome. A recent study demonstrated anaerobic enrichment of mucus from chronic rhinosinusitis patients promotes bacteria able to hydrolyse mucin into byproducts supporting *S. aureus* growth, though the species involved remain undefined.

To examine these interspecies relationships, we have established cross-feeding experiments to investigate whether nasal *S. aureus* strains can utilize metabolic by-products from other nasal microbiome species. We will present findings on which nasal species can support *S. aureus* growth on mucin and evaluate these relationships with an *in vitro* adhesion assay to assess how mutualistic breakdown of mucin influences host-*S. aureus* interactions at the epithelial cell surface.

A259

Mandrills and Microbes: Characterising the mandrill scent-gland microbiome and its potential role in olfactory communication

Daniel R Lewis ORCID iD[1,2], Leena Kerr ORCID iD[2], Sharon Kessler ORCID iD[3], Barthélémy Ngoubangoye[4,1], Joanna M Setchell ORCID iD[1,4]

[1]Durham University, Durham, United Kingdom. [2]Heriot-Watt University, Edinburgh, United Kingdom. [3]University of Stirling, Stirling, United Kingdom. [4]Centre Interdisciplinaire de Recherches Médicales de Franceville (CIRMF), Franceville, United Kingdom

Abstract

Olfactory communication conveys information about an individual through the release and reception of semiochemicals. Mammals harbour a diverse array of microorganisms, many of which have co-evolved to facilitate otherwise impossible metabolic pathways for the host. For example, the fermentation hypothesis holds that microbes inhabiting scent-glands digest secretions, producing odour signals. Olfaction is integral to primate communication, impacting behaviour and reproductive success. Mandrills (*Mandrillus sphinx*) are one of the few African monkey species to possess scent-glands, which produce odour that differs with age, sex, male dominance rank, group membership, and possibly individual identity. We aimed to determine the composition of the mandrill scent-gland microbiome and investigate how the bacterial composition differs with individual traits and states, and with glandular activity. We collected 121 skin swab samples from the scent-glands of mandrills living in a large, semi-free ranging colony in Franceville, Gabon. We used 16S rRNA amplicon sequencing and bioinformatic analyses with the QIIME2 pipeline to determine bacterial composition and diversity measures. Analysis is ongoing, but initial results indicate that the mandrill scent-gland is dominated by four phyla: Firmicutes (47.6%), Bacteroidota (16.5%), Actinobacteriota (12.9%) and Proteobacteria (11.9%). We also identified fermentative genera previously found in other mammalian scent-glands, including *Staphylococcus* (9.8%), *Prevotella* (9.1%), *Lactobacillus* (3.4%), *Corynebacterium* (0.4%), *Fusobacterium* (0.3%) and *Anaerococcus* (0.1%). This first description of the mandrill scent-gland microbiome will test the potential for microbiota to mediate signals used in selecting mates, the capacity for hosts to control their microbiome composition, and the co-evolutionary consequences of this symbiosis for primate communication.

A260

Understanding how community context drives virulence-associated traits in the Cystic Fibrosis pathogen *Pseudomonas aeruginosa*

Filza Masood, Siobhán O' Brien

Moyne Institute of Preventive Medicine, Department of Microbiology, School of Genetics and Microbiology, Trinity College Dublin, Dublin, Ireland

Abstract

The lungs of patients with Cystic Fibrosis (CF) are often chronically colonised by a plethora of microbial species, including the major CF pathogen *Pseudomonas aeruginosa (P. aeruginosa).* Herewith, when examining recalcitrant bacterial infections in CF, it is imperative to not only study pathogens in isolation, but rather, as part of ecological communities interacting at the species level. The successful invasion and robust virulence mechanisms of *P. aeruginosa* are influenced by members of the lung microbiome. Interspecies interactions can shape and often determine virulence evolution, mediated through synergistic and antagonistic behaviours. Moreover, species richness and composition act in concert to impact *P. aeruginosa* colonisation and may predict important clinical phenotypes – yet it is difficult in practice to tease apart the effects of diversity per se *versus* increased likelihood of encountering a key species at higher diversities. We use ecological framework to disentangle the effects that community richness and community composition have on colonisation resistance in the CF airways, and dissect how polymicrobial interactions shape *P. aeruginosa* virulence. By implementing a modified random partition design, we were able to identify members of an artificially assembled microbial community that affect *P. aeruginosa* invasion and virulence-associated secretions such as pyoverdine and pyocyanin. This work provides a novel way of understanding microbial interactions in a clinical context, by applying an ecological framework to artificially assembled communities. This will pave the way for future work applying this framework to more “natural” CF microbial assemblages that closely reflect different clinical statuses of the CF lung.

A261

Alternative to Invasive Bronchoscopy: Validating Tracheal Aspirates for Microbiome Profiling in Intensive Care Unit Pneumonia Patients

Parshatd Govindasamy ORCID iD[1], Jun Yeun Cho[2], Jiyoul Yang[2], Bumhee Yang[2], Sun-Hyung Kim[2], Ok-Jun Lee[3], Eung Gook Kim[4], Kalai Mathee[1], Giri Narasimhan[1,5], Yoon Mi Shin[2]

[1]Bioinformatics Research Group (BioRG), Florida International University, Miami, USA. [2]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Chungbuk National University Hospital, Chungbuk National University College of Medicine, Cheongju, Korea, Republic of. [3]Department of Pathology, Chungbuk National University Hospital, Chungbuk National University College of Medicine, Cheongju, Korea, Republic of. [4]Department of Biochemical and Medical Research Center, Chungbuk National University College of Medicine, Cheongju, Korea, Republic of. [5]Biomolecular Sciences Institute, Florida International University, Miami, USA

Abstract

Pneumonia causes significant mortality in intensive care unit (ICU) patients, yet traditional culture-based pathogen detection lacks sensitivity. While bronchoalveolar lavage fluid (BALF) provides optimal diagnostic yield, bronchoscopy is often contraindicated in critically ill patients. This study compares the respiratory microbiome profiles of paired tracheal aspirate (TA) and BALF samples from pneumonia patients in a tertiary hospital ICU (n=23, November 2019 - September 2022). Using 16S rRNA next-generation sequencing, we analyzed microbial diversity (Shannon Index), taxonomic composition, and differential abundance (edgeR). Results showed comparable diversity indices and microbial communities between TA and BALF samples, with TA successfully capturing key pneumonia-related microbial signatures. These findings validate TA as a reliable, less invasive alternative to BALF for respiratory microbiome analysis in critically ill patients, establishing the groundwork for future clinical applications.

A262

Integrative multi-omics uncovers dietary fibre-induced modulations in the structure and functional profile of the chicken cecal microbiota

Anum Ahmad[1], Kellie Watson[1], Farina Khattak[2], Laura Glendinning[1]

[1]The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom. [2]SRUC, Edinburgh, United Kingdom

Abstract

The chicken caecal microbiota plays an important role in fermenting dietary fibre into short-chain fatty acids that serve as an energy source for the host. This study employed a multi-omics approach to explore the effects of soluble and insoluble dietary fibre on the caecal microbiota and its functional profile in broiler chickens. Chickens were divided into five dietary groups: a basal diet (corn/soy), basal diet with 1% inulin, basal diet with 4% inulin, basal diet with 1% cellulose, and basal diet with 4% cellulose. DNA and RNA were isolated from caecal contents and sequenced using shotgun metagenomics and metatranscriptomics, respectively. We identified a significant impact of different dietary fibre on the taxonomic and functional profile of caecal microbiota in chickens. Microbial taxa capable of producing beneficial fatty acids and antioxidants were significantly more abundant in the inulin group, whereas fibre-degrading bacteria were more prevalent in the cellulose group. Additionally, more carbohydrate-degrading enzymes and sugar-transporting genes were identified in the inulin group. These findings highlight the presence of functionally specialized microbiota across different dietary groups. This study is currently ongoing, with further analyses in progress. In future analysis, we plan to integrate metaproteomics to better link transcribed genes with their protein products.

A263

Pumping iron: exploring a novel iron transporter in the early life microbiota member Bifidobacterium

Luke Acton ORCID iD[1,2], Cho Zin Soe[3], Magdalena Kujawska[2,4], Ayuki Shimpo[5], Peter Chivers[5,6], Lindsay Hall[1,2,3,7]

[1]Department of Microbes, Infection and Microbiomes, College of Medicine and Health, University of Birmingham, Birmingham, United Kingdom. [2]Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom. [3]Gut Microbes & Health, Quadram Institute Bioscience, Norwich, United Kingdom. [4]Intestinal Microbiome, School of Life Sciences, ZIEL – Institute for Food & Health, Technical University of Munich, Munich, Germany. [5]Departments of Biosciences, University of Durham, Durham, United Kingdom. [6]Departments of Chemistry, University of Durham, Durham, United Kingdom. [7]Norwich Medical School, University of East Anglia, Norwich, United Kingdom

Abstract

Iron availability in the gastrointestinal (GI) tract is limited, yet essential for microbial colonisation and persistence, particularly in within the context of the early life microbiome. While iron acquisition systems in GI pathogens are well-characterised, the mechanisms by which beneficial gut microbes, such as *Bifidobacterium longum*, obtain iron remain poorly understood.

In this study, we identify a unique energy-coupling factor (ECF) iron transporter in *B. longum* LH277, a strain isolated from a healthy breast-fed infant. Our preliminary findings reveal that this ECF transporter, identified through transcriptomic analyses under differential iron conditions, is part of an iron-responsive gene cluster and is well conserved in *Bifidobacterium longum.*

Functional assays demonstrate that heterologous expression of the ECF transporter in *E. coli* conferred a competitive advantage in iron deplete, simulated early-life microbial communities, suggesting a role for this system in iron acquisition under low-iron conditions. Analysis of the ECF protein subunits by AlphaFold (AF) identified candidate iron-binding residues in two subunits. Consistent with the AF analysis, we have demonstrated Fe(II)-binding to one subunit, further supporting the role of the ECF system in iron-acquisition.

This work lays the foundation for understanding the role of the ECF transporter in *B. longum* colonisation and persistence within the infant microbiota. Future investigations will explore how this system influences broader gut microbial ecosystems under varying iron conditions, providing insights with potential applications for probiotic interventions to lessen the side effects of iron supplementation in iron-deficient anaemic infants.

A264

The equine gut microbiome – understanding the bacteria generating horse power.

Joy Leng ORCID iD, Roberto La Ragione ORCID iD, Chris Proudman ORCID iD

University of Surrey, Guildford, United Kingdom

Abstract

Horses are an important part of both companion and farm animal industries within the UK. The racehorse industry alone employs over 85,000 people and is the second largest spectator sport in the UK. Horses are hindgut fermenters and gain most of their energy from the fermentation of their fibre-based diet by the bacteria that reside mostly within the large intestine. Until recently, little was known about the mostly anaerobic bacterial communities that reside within the gastrointestinal system of horses. However, advancements in DNA sequencing techniques have enabled the analysis of gut microbiome communities without the need for culturing. This approach has revealed that different compartments of the equine gastrointestinal system harbour different communities of bacteria. Researchers have also observed changes in the gut microbiome of horses linked with gastrointestinal disease, diet and aging.

Our recently published study utilised bacterial sequencing to better understand the development of the gut microbiome in the early life of Thoroughbred racehorses. The microbiome within the faeces of young foals' changes composition and increases in diversity until the foals are four months old, at which time it mirrors that of an adult horse. Low bacterial diversity and use of antibiotics when the foals were four weeks old were associated with higher chance of illness and poorer racing performance in the first four years of the foals' lives.

Further research is required to understand the functional importance of the bacteria within the gut and untangle causality between gut bacteria and a rage of health outcomes in horses.

A265

The probiotic potential of a native rumen microbe to shift the fermentation pathways in an *in vitro* model of the bovine rumen microbiota.

Alice Buckner[1,2], Laura Glendinning[1], Jolanda van Munster[2], Juan Palma Hidalgo[2], Mark Stevens[1], Mick Watson[2], Jamie Newbold[2]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]SRUC, Edinburgh, United Kingdom

Abstract

Microorganisms present in the stomach compartment, known as the rumen, of ruminants such as cattle and sheep, influence the ability of the host to digest its feed. The microbiota break down plant materials into energy-rich volatile fatty acids (VFAs) (primarily acetate, propionate, and butyrate), along with CO_2 and H_2, which are converted to methane. Methanogenesis is detrimental as it uses H_2 which could otherwise be used to make VFAs, and is exhaled and lost to the atmosphere, contributing to global warming. A higher abundance of the bacterial group *Prevotella* (now split into 7 clades) has been associated with lower methane producing animals, and the addition of isolates to rumen systems sporadically indicates they can shift fermentation away from methane production. In this research, four isolates of *Prevotella* (*Xylanibacter* clade) were added to an *in vitro* fermentation system with rumen fluid, and the methane, hydrogen, VFA, and ammonia levels measured over 24-hours. Although the analysis is ongoing, no differences have been observed in the methane and hydrogen levels produced between the treatments and controls. Though some statistically significant differences were observed in the major VFA levels, none were large enough to indicate biological significance. Further investigation of rumen microbes with potential anti-methanogenic properties is essential, with the huge potential impact this could have in reducing the livestock methane production of livestock and increasing digestion efficiency inconsistently evidenced in the literature. Further studies using a range of rumen microbes and administration methods are necessary to determine if this approach could be effective.

A266

Beyond Antibiotics: Examining the Effect of the Antivirulence Drug, Aurodox, on the Murine Microbiome.

Rebecca McHugh ORCID iD, Andrew Roe, Gillian Douce

University of Glasgow, Glasgow, United Kingdom

Abstract

Shiga Toxin-Producing *E. coli* (STEC) is an acute pathogen of the small intestine which is responsible for foodborne outbreaks of bloody diarrhoea. STEC infections are often associated with high morbidity and mortality rates due to the production of Shiga toxins which can initiate Haemolytic Uremic Syndrome (HUS) which is a major cause of acute renal failure in children. Unlike most bacterial infections, STEC cannot be treated with traditional antibiotics as they can lead to increased production of Shiga toxin and perturb the gut microbiota, increasing the risk of recurrent infection. Here, we investigate aurodox, a natural product of *Streptomyces goldiniensis,* as a potential anti-virulence therapy for the treatment of STEC. In previous work, to understand the compound's mechanism of action and suitability for repurposing as an anti-virulence compound, a multidisciplinary approach was used. Whole transcriptome analysis, cell infection and GFP-reporter assays revealed that aurodox transcriptionally downregulates the expression of the Type III Secretion System (T3SS)- an essential colonisation factor in EHEC. In recent work,we have established a *Citrobacter rodentium* + Stx murine model to study the efficacy of aurodox treatment against Shiga-toxin-positive infections. Furthermore, we have examined the effect of aurodox on the murine gut microbiota in both infected and mock-infected mice. This revealed that aurodox treatment induces specific changes to the microbiota including a bloom in the probiotic strain *Bifidobacterium animalis* without significantly effecting alpha diversity. These results nominate aurodox as a potential therapy for the treatment of STEC infections.

A267

Human skin bacteriophages infecting coagulase-negative Staphylococcus identify barriers to phage infection in *S. hominis*

Samah Alsaadi

University of Liverpool, Liverpool, United Kingdom. Taif University, Taif, Saudi Arabia

Abstract

Human skin is colonised by *Staphylococcus* species. Their relative abundance differs across skin sites, and the skin virome influences the dynamics of bacterial populations in the skin microbiome. This study aimed to investigate the diversity of skin bacteriophages infecting major skin coagulase-negative *Staphylococcus* spp (CoNS) and identify host range and barriers. Skin swabs were collected from 80 healthy volunteers at different body sites to isolate cutaneous phages that infect 5 selected *Staphylococcus* spp. A total of 40 phages were isolated and genome sequenced, corresponding to six genetic clusters with two clusters representing novel phages. Phage infection was qualitatively assessed using a wide host range of 140 strains across 8 different Staphylococcus species. We found that one novel phage, named øAlsa, had a greater ability to infect *S. hominis*, which was otherwise infected much less than other CoNS species using the 40 identified phages, indicating the presence of a defence barrier to limit phage infection. To further investigate the resistance pattern observed in *S. hominis* strains, a co-evolution experiment was conducted using *S. hominis* LIV1218 as a representative strain with the novel phage øAlsa_1. Phage-resistant mutants were isolated and sequenced, revealing different single nucleotide variants in the spoVG gene, which encodes a transcriptional regulator. The *S. hominis* spoVG mutant phenotypes showed increased biofilm formation and autolysis. The host range analysis of spoVG mutants showed discrete patterns of phage susceptibility compared to the wild type. We identify a link between the spoVG regulator, biofilm formation and phage resistance in *S. hominis.*

A268

Characterizing the Impact of Viral Infection on the Respiratory Tract Microbiome

Luke Haddock ORCID iD, Craig Thompson, Nicole Robb ORCID iD

University of Warwick, Coventry, United Kingdom

Abstract

The interaction between viral infections and the respiratory tract microbiome remains largely unknown, particularly how commensal microbes can protect against invading viruses. This project investigates the dynamics between commensal microorganisms and respiratory viral infections, focusing on the changes caused by viral infections in individuals of typical and non-typical microbiomes. Leveraging a biobank of over 70,000 swab samples from the UKHSA Lighthouse Labs, we will compare the microbiome diversity in healthy, infected, and uninfected individuals, with a specific analysis of those with inflammatory bowel disease (IBD), a condition associated with severe viral illness and altered microbiomes.

We will employ next-generation sequencing to profile bacterial changes, high-throughput microscopy for viral diversity, and mass spectrometry to assess shifts in protein expression. Association studies will identify commensal bacterial shifts along with gene and protein expression changes. Proteins from commensals showing differential expression will be purified and tested for their anti-viral effects on respiratory viruses, influenza and SARS-CoV-2 using plaque assays.

Ultimately, identifying microbial-derived inhibitors or activators of viral infection could lead to new therapies targeting viral infections as well as restoring microbiome balance in individuals with non-typical microbiomes.

A269

Exploring the impact of Flavoured E-Cigarette Liquids on Oral Microbiome Composition and Pathogenic Biofilm Formation in the Oral Cavity

Evie Longbottom, Daniel Ciuperca, Rachel Floyd, Athina Giannoudis, Sean Goodman ORCID iD

University of Liverpool, Liverpool, United Kingdom

Abstract

Originally marketed as a safer alternative to traditional cigarettes, e-cigarettes have grown in popularity. Recent reports show a concerning rise in youth vaping, promoted through appealing flavours and targeted marketing. Disruption of the oral microbiome through environmental and chemical changes linked to e-cigarette flavourings, may promote the growth of pathogenic microorganisms, leading to the progression of plaque, dental caries and inflammatory disorders such as gingivitis and periodontitis. Emerging evidence suggests that certain flavouring agents alter microbial composition and biofilm persistence posing unique risks to wider health.

This cross-disciplinary student-led study evaluated the impact of specific e-cigarette flavouring agents on bacterial growth within the oral microbiome, with an emphasis on biofilm formation and the promotion of pathogenic organisms. This project utilised a systematic approach to identify relevant studies in line with PRISMA guidelines and key flavouring agents that could be further tested through *in-vitro* studies. Quantitative microbiological methods were applied assessing bacterial growth and biofilm stability of several known pathogenic oral colonisers in the presence of e-liquid flavourings, comparing the effects to those of unflavoured liquids.

Preliminary findings from the systematic review and *in-vitro* experiments indicate that specific flavouring agents disrupt the oral microbiome, showing an increased capacity for biofilm formation, posing potential risks to biofilm integrity and enhancement in growth of cariogenic microorganisms. This study highlights the need for further investigation into the long-term effects of e-cigarette use on oral and systemic health and underscores the importance of education and public health initiatives to mitigate the rising vaping-related health burden.

A270

Assessing the impact of antibiotic administration on prophages in the preterm infant gut

Jasmine Jessop[1], Wen Chyin Yew[1], Nicholas D. Embleton[2], Janet E. Berrington[2], Christopher J. Stewart[3], Darren Smith[1], Andrew Nelson[1]

[1]University of Northumbria, Newcastle Upon Tyne, United Kingdom. [2]Newcastle Neonatal Service, Newcastle Hospitals NHS Trust, Newcastle Upon Tyne, United Kingdom. [3]Newcastle University, Newcastle Upon Tyne, United Kingdom

Abstract

Previously, research has indicated that the majority of bacteriophages identified in the gut are passively replicated within their bacterial hosts genome as prophages. These prophages have been found to harbor virulence factors and antibiotic resistance genes that may confer advantages to the host bacteria. Environmental changes in the gut can cause bacterial stress, prompting the induction of these prophages into the lytic cycle. This transition could facilitate the horizontal gene transmission to other susceptible hosts.Notably, antibiotics can provoke an SOS-response in gut lysogens, inducing prophages which can then contribute to the horizontal transmission of virulence genes.

S. epidermidis is one of the pathogens associated with late onset sepsis, a disease that affects preterm infants after 72 hours of life. Following preterm birth, antibiotics are routinely administered to try prevent diseases like late onset sepsis, a disease which is considered to be one of the leading causes of morbidity and mortality among neonates. The aim of this study is to understand the impact of clinically relevant antibiotics on strains of coagulase negative Staphylococci (CoNS) isolated from preterm samples, collected from the Neonatal Intensive Care Unit (NICU) in the RVI hospital, Newcastle upon Tyne. The samples were exposed to the minimum inhibition concentration of each antibiotic, and the 'lysate' was screened against multiple CoNS hosts to determine infection. Additionally, each lysate was sequenced to identify propagated phage without naïve hosts. The resulting data showed that various antibiotics were able to induce different prophage.

A271

When Friend Becomes Foe: Epidemiology of Pathogenic Swine-derived *Streptococcus suis* on Irish Farms

Emmanuel Osei[1,2,3], Kate O'Mahony[1,3], Reuben O'Hea[2], Margaret Wilson[4], Aine O'Doherty[4], Jennifer Mahony[1,2], John Kenny[1,3]

[1]APC Microbiome Ireland, Cork, Ireland. [2]School of Microbiology, UCC, Cork, Ireland. [3]Teagasc Food Research Centre, Moorepark, Ireland. [4]Department of Agriculture, Food and the Marine, Backweston, Ireland

Abstract

Streptococcus suis is a Gram-positive pathobiont that colonises the nasopharynx of pigs. During stressful events such as weaning when specific maternal antibodies are low, virulent strains can proliferate within herds causing infections such as meningitis, endocarditis, septicaemia and pneumonia leading to significant animal welfare and economic burden in the Irish swine industry. In Ireland, *S. suis* is the most common cause of meningitis in pigs. Furthermore, reports of zoonotic transmissions are becoming increasingly common globally. However, to date, there are few reports on epidemiology of the pathobiont on Irish farms with zero to few sequence information on *S. suis* on databases such as PubMLST and GenBank. This study aimed to establish baseline information on S. suis infections on Irish farms from 2005–2022.

A272

Limiting oral iron in weaning piglets promotes beneficial gut bacteria, immune development and favourable metabolite production without inhibiting weight gain

Chloe Callow ORCID iD[1], Zeynep Hayirli ORCID iD[1], Daniel James ORCID iD[1], Simon Andrews ORCID iD[2], Arnoud van Vliet ORCID iD[3], Roberto La Ragione ORCID iD[3,4], Marie Lewis ORCID iD[1]

[1]Department of Food and Nutritional Science, Harry Nursten Building, University of Reading, Whiteknights, Reading, United Kingdom. [2]School of Biological Sciences, Health and Life Sciences Building, University of Reading, Reading, United Kingdom. [3]School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom. [4]School of Biosciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom

Abstract

Abrupt early weaning in piglets contributes to post-weaning diarrhoea (PWD), the primary welfare and economic issue within pig farming. Prophylactic zinc oxide (ZnO) has been used to limit PWD, but is now banned and there are no reliable alternatives available. Piglets are susceptible to iron-deficiency anaemia, and thus receive injected iron post-birth, and high dietary iron at weaning. However, increased dietary iron provides favourable environments for enteropathogens which can exacerbate PWD. Since piglets are not anaemic at weaning, we hypothesised that iron could be reduced without adverse consequences. To explore this, eighteen 28-day old piglets were litter-matched into three groups (n=6): high iron (150mg/kg bodyweight) with ZnO; high iron and low iron (69mg/kg feed). Systemic haemoglobin and ferritin were routinely quantified, while terminal faecal and urine samples were analysed using 16S rRNA gene sequencing and SPME-GC/MS to assess microbiota and metabolites, respectively. Bioinformatics analyses was conducted in QIIME2, and differential abundance analysis (DESeq2) was conducted in R. Frozen colon sections were analysed using 4-colour fluorescence immunohistology to quantify CD45 (T-lymphocytes), CD172/Sirpa (dendritic cells), MHC II and capillary endothelium expression. Low-iron was associated with increased *Faecalibacterium* (p<0.01), higher beneficial urinary metabolites and reduced *Bilophila* (p<0.01), compared to high-iron feed. Piglets treated with ZnO had increased co-expression of antigen-presenting complex ($CE^+MHCII^+CD172^+CD45^+$; p<0.01), reduced *Desulfovibrio* (p<0.01), but increased *Pseudomonas* (p<0.01). Importantly, growth and iron status remained unaffected by treatment. Reductions in iron benefitted the gut microbial profile and immunity without impacting weight-gain, iron storage, thus providing a novel strategy to reduce PWD.

A273

Exploring the impact of the respiratory tract microbiome on epithelial integrity – do commensals vs. pathogens have differential influences on the function of tight junctions?

Lillie Purser[1,2], Mohammadali Ramsheh[1], Sean Brennan[1], Bara Sinz[1], Richard Haigh[1], Christopher Brightling[1,2], Julie Morrissey[1,2]

[1]University of Leicester, Leicester, United Kingdom. [2]NIHR Leicester Biomedical Research Centre, Leicester, United Kingdom

Abstract

There is a strong association between chronic obstructive pulmonary disease (COPD) and bacterial infection, with 50% of disease exacerbations being directly linked with infection by bacteria including *Moraxella catarrhalis*. Whether a bacterial infection is a cause or consequence of disease exacerbation remains unclear. Our recent study elucidated that considering only pathogens when discussing the bacterial link with COPD provides a limited view of the overall diversity of the respiratory tract. There is now evidence that the abundance of specific bacterial genera in the lung microbiome shifts as COPD severity increases - as COPD symptoms worsen, the abundance of commensal *Prevotella spp.* decreases, while the opportunistic pathogen *Moraxella spp.* abundance increases. These shifts in the diversity of the microbiome are associated with lower expression of genes promoting epithelial barrier integrity, including those which encode tight junction proteins. Tight junctions are important structures for maintaining epithelial integrity and as such are an important marker of lung health. The association between *Prevotella spp.* and *Moraxella spp.* abundance, and the expression of tight junction proteins implies that the composition of the microbiome might be able to directly affect epithelial integrity, suggesting a direct link between the microbiome and disease outcomes.

The relationship between respiratory microbiome commensals and pathogens on the integrity of the respiratory epithelium was explored using Calu-3 monolayers grown at the air-liquid interface. Findings show that *P. melaninogenica* has a weaker potential to damage epithelial integrity than *M. catarrhalis*, measured by trans-epithelial electrical resistance (TEER) and confocal imaging of fluorescently labelled occludin.

A274

The effect of air pollution on wild *Bombus terrestris* gut microbiome.

Hannah Sampson ORCID iD, Alun Jones, Eamonn Mallon, Julian Ketley, Julie Morrissey

Leicester, Leicester, United Kingdom

Abstract

Air pollution is the world's largest environmental health risk, detrimental to human health and a significant risk to wildlife and ecosystems globally. Air pollution is pervasive in our atmosphere and pollution particulates accumulate on bee's bodies and food stores. Yet, there is limited knowledge of how pollutants might detrimentally affect bees and to what extent. A vital component of bee health is their beneficial core gut microbiota, these specialised core bacteria have co-evolved with their host for millions of years and are found in honeybees and bumblebees worldwide.

Our previous studies were the first to show that major air pollutant, black carbon, changes the behaviour of host associated bacteria. We discovered that black carbon exposure disrupted the gut microbiome of important UK pollinator, the buff-tailed bumblebee (*Bombus terrestris*), reared in laboratory conditions. Providing the first evidence that particulate pollution black carbon, disrupts the core *B. terrestris* gut microbiome.

We sampled wild *B. terrestris* from seven differently polluted UK sites and investigated the effects of environmental factors (air pollution and temperature) on their gut microbiome. We found that wild *B. terrestris* had a highly diverse gut microbiome composition, containing many non-core taxa, and found significant changes in gut microbiome diversity and taxon abundance between differently polluted sites. Importantly, environmental factors, such as pollution level, had distinct predictive relationships with core and pathogenic bacteria. Together these data highlight the importance of studying the impact of pollution on the bee gut microbiome and its underexplored risk to insect pollinator health.

A275

Structural integrity of fava bean cotyledon cells modulates the in vitro colonic fermentation and potential health benefits

Glória Máté-Koncz, Peter Ryden, Natalia Perez-Moral, Catherine Booth, Hannah Harris, Emad Shehata, Frederick Warren, Arjan Narbad, Cathrina Edwards

Quadram Institute Bioscience, Norwich, United Kingdom

Abstract

Regular pulse consumption is associated with reduced risk of type 2 diabetes, obesity and colon cancer but the underlying mechanisms are poorly understood. The structural composition of pulses and their influence on digestion may help elucidate mechanisms. Pulses such as fava beans (*Vicia faba L.*) comprise cotyledon cells, in which starch is surrounded by the protein matrix and encapsulated by the plant cell wall (dietary fibre). When cooked, this structure is highly resistant to digestion in the upper gastrointestinal tract, allowing a portion of the starch to reach the colon, where undergoes fermentation along the dietary fibre by the microbiota, releasing short-chain fatty acids that provide health benefits to the host.

This study aimed to investigate the *in vitro* colonic breakdown of fava bean cells with varying structural integrity to explore how structural composition may influence fermentation behaviour and potential health benefits. The hypothesis was that intact cells would ferment more slowly than the broken cells, potentially offering greater benefits to the host. The fermentation experiments were conducted using batch models with fresh healthy human faecal materials for either 24- or 48-hour periods. Metabolite production and microbial composition shifts were analysed with untargeted nuclear magnetic resonance and metagenome sequencing at multiple time points. The results indicated that structural differences lead to distinct microbial compositions and metabolite profiles.

This study demonstrated that variations in the pulse cell structure significantly influences the digestion pathways, highlighting the role of food structure in modulating health benefits and supporting dietary strategies for microbiome modulation.

A276

The cooperation inside us: investigating bacterial cooperation in the human gut

Zohar Katz, Laurence Belcher, Anna Dewar, Stuart West

University of Oxford, Oxford, United Kingdom

Abstract

The bacteria in our gut are thought to play a major role in our development, behaviour and disease. Rather than acting in isolation, bacteria are extremely social. Cells secrete molecules which benefit nearby cells, with this cooperation allowing the invasion of hosts and acquisition of nutrients. However, the evolution of cooperation is not well understood in our gut communities. Particularly, it is unclear what kind of selection pressures influence cooperation in gut bacterial communities. To explore this, we analysed gut metagenome data from 231 humans. We calculated the species' relatedness (how similar strains are to one another), polymorphism and protein divergence of genes encoding for cooperative and private traits. We found that the number of cooperative genes varied greatly between species, but the proportion of cooperative genes increases as species' relatedness increases. We then examined whether genetic diversity in these genes was correlated with the relatedness of species. Evolutionary theory suggests that genes for cooperation should be under relaxed selection, which should mean that **diversity in genes for cooperative relative to private traits would increase as relatedness decreases.** However, opposite to these predictions, we found that species with higher relatedness showed higher relative polymorphism in genes for cooperative compared to private traits. This suggests that other factors at play. For example, selection on cooperation in the gut might be different compared to other systems studied so far. Further investigation and statistical analysis are under way to find out.

A277

A look into the microbiome of diabetic foot ulcers: How does it impact patient outcomes?

Jontana Allkja ORCID iD[1,2,3], Sakcham Bairoliya[4,5], Ahmed Bakri[1], Jason L. Brown[1], Kelly Newby[6], Andrew Gilmour[2], Craig Williams[7], Cao Bin[4,5], Gordon Ramage[2]

[1]University of Glasgow, Glasgow, United Kingdom. [2]Glasgow Caledonian University, Glasgow, United Kingdom. [3]University of Liverpool, Liverpool, United Kingdom. [4]Singapore Centre for Environmental Life Sciences Engineering, Singapore, Singapore. [5]Nanyang Technological University, Singapore, Singapore. [6]University Hospitals of Morecambe Bay NHS Foundation Trust,, Kendal, United Kingdom. [7]Lancaster Royal Infirmary, Lancaster, United Kingdom

Abstract

Diabetic foot ulcers (DFUs) are a result of poor circulation and neuropathy in lower limbs coupled with a compromised immune system. The role of the microbiome in DFUs is becoming increasingly evident, with some suggesting it could be a deciding factor in healing. In our study, we investigated the microbiome of 128 patients at the Royal Lancaster Infirmary and surrounding clinics, to understand their microbial composition and identify any trends related to healing. Swabs were collected across several visits resulting in 349 total samples. Data on different clinical information was also collected for each visit. 16S sequencing was performed using the Oxford Nanopore MinION sequencing platform. Bioinformatics analysis was performed to evaluate Alpha and Beta diversity, as well as microbial interaction networks.

Preliminary analysis of the data shows key pathogens like *S. aureus* and *P. aeruginosa* are present, but there are no clear distinctions in communities based on wound grade and stage, or healing outcomes. There are no significant differences in alpha and beta diversity based on wound grade and stage. However, for positive outcomes complex microbial networks appear, containing negative interactions suggesting that key node species might be keeping pathogens under control. These interactions are broken in the negative outcomes. Further analysis of our data is necessary to identify potential factors contributing to this. These results could potentially help guide future treatment choices. Identifying key microbiome species necessary for healing could pave the way towards the development of alternative forms of wound management such as probiotics.

A278

Application of Clinical Metagenomics for Accurate Detection of Community-acquired Pneumonia (CAP) in Intensive Care Patients: A Pilot Study

Mahboobeh Behruznia ORCID iD, Nicola Cumley, Alan McNally

Microbes, Infections and Microbiomes, University of Birmingham, Birmingham, United Kingdom

Abstract

Background
Community-acquired pneumonia (CAP) is a major global cause of mortality, morbidity, and high healthcare costs. Rapid and accurate diagnostics that can distinguish between viral and bacterial infections are critical to reduce unnecessary antibiotic use and enable pathogen-directed treatments. Clinical metagenomics offers a promising solution by rapidly identifying all pathogens in clinical samples.

Methods
Samples including nasopharyngeal swabs, sputum, and pleural fluid were taken from CAP patients and control individuals. Human depletion was performed using kits or no depletion, followed by total nucleic acid extraction. Viral metagenomics was conducted using SMART-9N, and library preparation and sequencing were performed with Oxford Nanopore Technology. Sequencing results were compared to clinical microbiology data where available.

Results
Metagenomics identified several key CAP pathogens, including those missed by conventional diagnostics methods (*Streptococcus spp., Klebsiella pneumoniae, Legionella pneumophila*, *Tropheryma whipplei, etc*). Importantly, the analysis also detected antibiotic-resistance genes. No false-positive viral detections were observed, but three false-negative results occurred: *Stenotrophomonas maltophilia* and *Candida albicans* by sputum culture, and *Mycoplasma pneumonia* by BioFire® respiratory panel.

The choice of depletion method significantly impacted bacterial read counts. Notably, samples treated with saponin and Molysis™ yielded significantly higher bacterial reads compared to no depletion.

Discussion
This pilot study demonstrates the potential of clinical metagenomics to detect pathogens, including those overlooked by standard culture methods, and to identify resistance genes, guiding appropriate treatments. Refining host depletion methods, improving accuracy in low-biomass samples, and optimising protocols will enhance its clinical utility and diagnostic precision for targeted CAP treatment.

A279

Elucidating the molecular mechanisms that commensal bacteria utilize to antagonize Tuberculosis infection

Oluwadunmininu Okude, Jorge Gutierrez-Merino ORCID iD, Suzie Hingley-Wilson ORCID iD

University of Surrey, Surrey, United Kingdom

Abstract

Tuberculosis (TB) is an insidious, serious infectious disease caused by different species of the *Mycobacterium tuberculosis* complex (MTC) posing a significant burden to both humans and animals worldwide. Therefore, there is an urgent need for more effective control measures against TB. One potential alternative could be the exploitation of the host commensal bacteria to impede the complicated process by which MTC strains evade host immune defences. We hypothesise that commensal bacteria possess unique molecular mechanisms associated with pathogen clearance, protecting the host from TB infection.

Through co-culture studies, we have identified three *Lactobacillus* species, *L. plantarum*, *L. salivarius*, and *L. casei*, that are capable of inhibiting the growth of MTC, and this antibacterial activity seems to be related to their ability to overexpress antimicrobial peptides (AKA bacteriocins). On the other hand, flow cytometry and ImageStream analysis have let us find out that all the *Lactobacillus* species are phagocytosed by monocytes and macrophages, resulting in a decrease in the uptake of MTC and the formation of apoptotic bodies. Our next objective is to determine the mechanisms that lactobacilli utilize to antagonize MTC, extra- and intra-cellularly.

Overall, our results reinforce the importance of the newly emerging concept of commensal bacteria as a potential biological tool to combat the establishment and progression of intracellular pathogens such as MTC, providing novel insights into microbiome strategies dedicated to fighting TB in animals and humans.

A281

Isolation and identification of antibiotic resistant bacteria and genes from soil. The effect of animal manure use as a fertiliser on antimicrobial resistance in an Irish beef and sheep farm.

Brian Joyce, David O'Connell, Marta Martins

Trinity College Dublin, Dublin, Ireland

Abstract

Introduction:

Antimicrobial resistance (AMR) is a public health issue affecting many areas including agriculture. In farms, animal manure can contain antibiotic resistant genes, antibiotic resistant bacteria and undegraded antibiotics. Due to the use of animal manure as a fertiliser and its deposition on soil, there are fears that AMR could spread through the food chain from animals to the environment. Therefore, the impact of animal manure spreading on AMR needs to be investigated.

This study aims to; take soil and animal manure samples before and after animal manure spreading; extract DNA and characterise the taxonomic and AMR gene profile using shotgun sequencing and isolate resistant bacteria to investigate multidrug resistance (MDR) and resistance mechanisms.

Methods:

Animal manure samples were taken before spreading and soil samples taken before, 2 weeks, 1, 2 and 3 months after manure spreading. Bacterial DNA was extracted from soil and manure and sent for shotgun sequencing.

Antibiotic resistant bacteria were isolated by diluting soil samples in phosphate buffered saline solution and inoculating directly onto antibiotic media.

Results:

36 colonies resistant to ampicillin, ertapenem, ciprofloxacin, colistin, chloramphenicol, trimethoprim, amikacin and tetracycline were isolated so far and are being tested for multidrug resistance. These results highlight the presence and dissemination of MDR on farms.

Conclusion:

This work investigates the effects of animal manure use as a fertiliser on the spread of AMR on farms. Shotgun sequencing data will reveal the impact of animal manure spreading on the microbial taxonomic and AMR gene profile of soil in agricultural fields.

A282

Understanding the role of phytogenic feed additive-modulated microbiomes on dairy cow health

Freya Bowden ORCID iD[1], Sharon Huws ORCID iD[1], James Pickup ORCID iD[1], Fernanda Godoy-Santos ORCID iD[1], AHV Personnel ,[2], Linda Oyama ORCID iD[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]AHV international, Zwolle, Netherlands

Abstract

Since the EU banned antibiotic feed additives for livestock production in 2006, the popularity of plant-based alternatives (phytogenic feed additives) has grown rapidly. While these have been implemented globally and demonstrated benefits in animal health and production, especially in dairy cow production, little is understood about how these phytogenic compounds interact with the microbial populations in the gut of dairy cows to produce these benefits, particularly in promoting udder health and reducing reliance on antibiotics. Here, we evaluated the susceptibility of core ruminal bacterial isolates (n=13), including *Prevotella* and *Bifidobacterium* species, to phytogenic feed additive ingredients (n=49), such as plant extracts and essential oils, using a modified high-throughput agar-well diffusion method. Test compounds showing activity in the agar screen (n=43), were further tested by microbroth dilution susceptibility assays adapted for fastidious anaerobic rumen bacteria. Allium derivates used in industry-implemented formulations and oregano essential oils demonstrated the highest levels of broad-spectrum antimicrobial activity with their MIC values ranging from 0.156-<0.078%V/V. This study represents the first comprehensive examination of the microbial activity of industry-implemented plant-based additives on ruminal anaerobic bacteria and provides foundational data on the antimicrobial effects of conventional antibiotics on rumen gut bacteria. Further characterisation of the phytogenic feed-modulated microbiome via meta-omic *in vitro* batch fermentation and *in vivo* studies is ongoing. These findings not only provide insight into the potential modulatory properties of plant-based alternatives on the rumen microbiota but highlight a promising approach for replacing antibiotics and reducing antimicrobial resistance (AMR) whilst enhancing animal health and productivity.

A283

Odours at the interface of host-microbiome interaction

Nikita Mehta, Ritika Siddiqui, Varsha Singh

University of Dundee, Dundee, United Kingdom

Abstract

The microbiome, a community of microbes present in the body, is an essential factor that plays a role in mediating the health of animals. The dysbiosis in the gut microbiota leads to various diseases in animal hosts. One factor that is believed to be important in regulating host-microbiota interaction is bacterial-derived volatile organic compounds including alcohols, acetates, short-chain fatty acids, and branched-chain fatty acids amino acids. It is well documented that several conditions are associated with a unique odour. Still, there is scant evidence of how microbes and odours are influencing the animal's metabolism and physiology. In our lab, we use two model organisms to identify their gut odours: one is nematode *Caenorhabditis elegans*, a small roundworm, and the second is a rodent mouse model. We identified the odours from the gut microbiota of small roundworm *C. elegans* and mouse models. We have determined the neuronal basis of odour perception in *C. elegans* for some of the odours and continue doing it. This work, in the long run, will further help identify odour as biomarkers that are linked with ageing and specific metabolic, infectious and neurodegenerative diseases and eventually benefit human society.

A284

Coated vs Uncoated Calcium Peroxide for Methane Mitigation in Rumen Fluid

Alison Hall ORCID iD[1], Paul Abberton[2], Sandra O'Connor[1], Andrew Bartle[1], Vincent O'Flaherty[1]

[1]University of Galway, Galway, Ireland. [2]GlasPort Bio, Galway, Ireland

Abstract

As part of the Climate Action Plan, Ireland has made a promise to reduce greenhouse gas emissions by 51% by the year 2030. As enteric methane emissions from cattle accounted for 19% of Ireland's total greenhouse gas emissions in 2023, the Irish government has placed pressure on the agriculture sector to reduce their emissions by 25% by 2030.

Methanogens, which are anaerobic archaea, are responsible for the production of methane in the rumen of cattle and other ruminants. Methane mitigation strategies such as the supplementation with 3-nitrooxypropanol (3-NOP), seaweed, lipids and essential oils have recently grown in popularity. Another body of research surrounding the effectiveness of calcium peroxide (CaO_2) in inhibiting the production of methane *in vitro* and *in vivo* is also developing. As a potent oxidising agent, CaO_2 releases oxygen into the rumen environment thereby shutting off the methane production carried out by methanogens.

Inspired by encapsulation technologies used in control release fertilisers (CRF), the goal of this research is to investigate the effectiveness of different coating materials to encapsulate CaO_2 to prolong its methane mitigation power, with a focus on carbohydrate-based materials. The results from preliminary experiments conducted in batch systems using rumen fluid have demonstrated that coated CaO_2 reduces methane production over a prolonged period of time compared to uncoated, with carboxymethyl cellulose performing the most favourably.

Future directions for this research involve investigating the impact of a CaO_2 feed additive on the rumen microbiome and any consequences this may present for the animals themselves.

A285

"Unmasking the microbial interactions which underpin successful ruminant methane mitigation *via* novel oxygen-releasing feed additives".

Alison Graham ORCID iD[1,2], Camilla Thorn[2], Anna Trego[1], Sinead Waters[1], Umer Ijaz[3], Vincent O'Flaherty[1,2]

[1]University of Galway, Galway, Ireland. [2]GlasPort Bio, Galway, Ireland. [3]University of Glasgow, Glasgow, United Kingdom

Abstract

Ruminant livestock contributes significantly to global methane (CH_4) production and its mitigation is of utmost importance. Feed additives represent a cost-effective means of achieving this. Previous research demonstrated that slightly elevating the rumen oxidation reduction potential (ORP) using oxygen-releasing feed additives serves to hinder methanogenesis. This is due to the niche specialisation of methanogens, which are typically only active at ORPs below -300 millivolts. *In-vitro* assessment of these compounds, including both calcium and magnesium peroxide (CaO_2, MgO_2), and encapsulated liquid H_2O_2 for controlled, slow release has demonstrated effective CH_4 mitigation potential, with consistent CH_4 reductions of >50% observed. Encapsulated formats of CaO_2 and MgO_2 offer potential feasibility as a CH_4 mitigation feed additive solution in both intensive and pasture-based production systems. However, the influence these oxygen-releasing compounds have on the rumen microbiome, specifically what might be occurring when hydrogen (H_2) or carbon dioxide (CO_2) are diverted away from ruminant CH_4 production remains unclear. This study focuses on the impacts of ORP modulating compounds on *in-vitro* rumen microbial communities. Nucleic acids were co-extracted from rumen fluid and cDNA was subsequently synthesized from the RNA. Amplicon sequencing of the 16S and 18S genes was performed on all samples (n=64) comprising inoculum (time zero), CaO_2, MgO_2, liquid H_2O_2, encapsulated liquid H_2O_2, and controls, over the course of a 21-day RUSITEC trial. Microbial community dynamics were integrated with process data and revealed the extent to which ORP can alter the rumen microbial community, thus elucidating the microbial mechanisms which underpin the CH_4 reductions observed.

A286

Comparative analysis of stool stabilization methods for the recovery of enveloped and non-enveloped RNA viruses

Roop Dhillon[1], Willem van Schaik[1], Richard Horniblow[1], Sarah Harding[2]

[1]University of Birmingham, Birmingham, United Kingdom. [2]Defence Science and Technology Laboratory, Porton Down, United Kingdom

Abstract

Stool stabilisation solutions are used to collect stool samples for metagenomic studies. These methodologies aim to stabilise the composition of the gut microbiota, to allow storage and transport, under conditions where ultra-low-temperature freezing is unavailable. In this study, we aimed to determine whether widely used stool stabilisation solutions could also be used for the recovery of RNA viruses. This group of viruses includes important enteric pathogens, including rotavirus and norovirus.

To investigate the stability of RNA viruses in stabilisation solutions, we used two bacteriophage - the enveloped *Pseudomonas syringae* phage Phi6 and the non-enveloped *Escherichia coli* phage MS2 - as proxies for human RNA viruses. We assessed phage recovery rates from three different stool stabilisation solutions OmniGene GUT systems OMR-200 and OMR-205 (DNA Genotek) and DNA/RNA Shield (Zymo Research). For each sample the phage were stored at three different storage temperatures (-20°C, 4°C and room temperature) and phage recovery was measured by quantitative PCR for 7 days.

We found that OMR-205 was a poor storage system for the phage preparations. Storing phage solutions at -20°C, in all buffers, resulted in no significant differences in yield between timepoints; equivalent to incubating in DNA/RNA Shield at 4°C. OMR-200 was poor at preserving RNA integrity at temperatures above -20°C, stability for the phage shown to be worse than the PBS control at all timepoints after 1 day at these temperatures. This work highlights considerations for future gut microbiome studies that seek to characterise interactions with enteric RNA viruses.

A288

Enhancing Taxonomic Resolution in Ruminant Microbiomes: A Dual Database Approach

John-Paul Wilkins ORCID iD, Chris Creevey

Queen's University, Belfast, United Kingdom

Abstract

Ruminant-associated microbiomes play a crucial role in the health, production efficiency, and environmental impact of global agriculture. These microbial communities are typically characterised by sequencing a specific genomic region, the 16S rRNA gene, which is nearly universal in prokaryotes. By referencing well-established databases, such as Greengenes2, we can enhance our understanding of these complex communities. However, while these databases provide accurate taxonomic identification, they often lack the resolution needed for niche-specific applications.

Our approach leverages sequence similarity to classify sequences against both broad, generic databases and custom-built databases tailored to specific niches. This dual strategy allows for more precise taxonomic placement of microorganisms. A key objective of our research is to consolidate multiple datasets and integrate this knowledge to gain insights into rarely observed microbes. Our results show potential links to host genome heritability, developing insight into the holobiont concept, where the host and its associated microbiota function as a single ecological unit.

Session : Exploring Hidden Threats: Knowledge Expansion of Understudied Bacterial Pathogens

A289

Resolving the Role of Biofilms in the Failure of Voice Prosthesis Implants

Louise Bolton[1], Liam Rooney[1], Rebecca McHugh[2], Pamela Thompson[3], Sarah De Blieck[3], Jan Stanier[3], Catriona Douglas[3], Gail McConnell[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom. [3]Queen Elizabeth University Hospital, Glasgow, United Kingdom

Abstract

Silicone tracheoesophageal valves are fitted in patients following surgical removal of advanced head and neck cancers; however, they are routinely colonized by biofilms in vivo which leads to high failure rates. The failure rate of voice prosthetics (VPs) leads to the patient's dependency on regular clinical intervention to replace failed VPs, and frequent use of high-dose antimicrobials to control the colonization of the implant. Despite these burdens, the composition and impact of colonizing pathogens on VP integrity are poorly understood.

We present workflows to determine the bioburden on explanted VPs to better understand the role of microbial pathogens in the high failure rate of tracheoesophageal implants. We used multimodal reflection and fluorescence confocal microscopy to map the surface of the VP and reveal the presence of bacterial and fungal biofilms. The bioburden was determined by quantifying the levels of different biofilms on the surface, and deterioration of the VP surface was monitored by reflection imaging. Then, the diversity of the bioburden was quantified by amplicon sequencing of gDNA isolated directly from the surface of a subset of VPs.

We show that VPs were colonized by both fungal and bacterial communities, which form discrete biofilms over the surface. The fungal and bacterial species identified confounds previous research and established clinical practice. The new insights show that complex polymicrobial biofilms are responsible for implant failure. Furthermore, our findings indicate that more tailored antimicrobial therapies and improved design and manufacture of the implants are required to lower the bioburden of VPs.

A290

Simple Living: Gene Expression In Minimal Bacteria

David Forrest ORCID iD, David Grainger

Institute of Microbiology and Infection, University of Birmingham, BIRMINGHAM, United Kingdom

Abstract

Our understanding of bacterial transcription comes from decades of research using the model organism *Escherichia coli*. However, it is increasingly apparent that what is true for *E. coli* is not necessarily true for other bacteria, making it critical to understand transcription in other genera.

One such genus is *Mycoplasma.* This understudied genus encompasses the simplest and smallest free-living organisms that arose through a severe reductive evolution. This streamlining has left *Mycoplasma* obligate parasites that cause chronic diseases in humans and livestock. *Mycoplasma* harbour incredibly minimal AT-rich genomes, which encode few regulatory transcription factors. Whilst these minimal genomes can provide the blueprints for cellular life, their AT content and loss of regulatory transcription factors challenges our understanding of transcription.

To begin to understand how the simplest bacteria successfully regulate gene expression on AT-rich DNA, we have conducted preliminary transcriptomic analysis on *Mycoplasma genitalium*. Using cappable-seq and term-seq, we have mapped global transcription start and termination sites. The major bacterial σ factor recruits RNA polymerase to DNA by recognising promoters made up of the -35 and -10 elements. The consensus promoter derived from our data suggests *M. genitalium* has lost the -35 element but has strong conservation of the -10 element. The optimal -10 element has the consensus 5'-TATAAT-3'. To understand if and how *M. genitalium* σ factor specifically recognises -10 elements in a 69% AT-rich genome, we have begun recombinant expression and purification of *M. genitalium* RNA polymerase and σ factor for use in an *in vitro* approach.

A291

Salmonella Typhi and Host Metabolism: Insights from primary Monocyte Infections

Sudip Dey[1], Swarnali Chakraborty[1], Animesh Gope[1], Santasabuj Das[2]

[1]ICMR - NATIONAL INSTITUTE FOR RESEARCH IN BACTERIAL INFECTIONS, KOLKATA, India. [2]ICMR-National Institute of Occupational Health, Ahmedabad, India

Abstract

Salmonella is a type of bacteria that can cause foodborne illness, commonly known as salmonellosis in tropical and subtropical region particularly in South Asia, parts of Africa, and some regions of Latin America. Till now there are vivid studies on Salmonella Typhimurium pathogenesis . But there are only few studies on human specific intracellular pathogen Salmonella Typhi (Ty2) . Salmonella Typhi mainly invade through M-Cells and spread through systemic circulation through different migratory cells . Monocytes ,Macrophages ,Dendritic cells been the niche of S. Typhi .Here we show that due S. Typhi infection Hexokinase(HK2) level alteration in human primary Monocytes. Here, we have found p-GSK-3B involvement in Hexokinase-2 mitochondrial localization difference in case of S. Typhi infection . Then in the upstream p-PKA/C-cAMP axis involved. So ,we are expecting the involvement of GPCR signalling ,research in going on to find the receptor.Salmonella is decreasing phagosomal acidification by regulating host glycolysis through mitochondrial localization of HK2 and increasing it’s survivality in human primary Monocytes.We investigated the role of vATPase in Typhi infection and found the expression reduction.Here we have also found the involvement of glycolysis in vATPAse reduction.Thus we found the role of S. Typhi in glycolysis regulation and phagosomal acidification reduction which ultimately be benificial for it's survival.

A292

Global rise and fall of globally disseminated Campylobacter spp. lineages

Madison Goforth ORCID iD, Ben Pascoe, Frances Colles, Samuel Sheppard

University of Oxford, Oxford, United Kingdom

Abstract

Campylobacteriosis is the leading bacterial cause of diarrheal disease globally, with disproportionately high prevalence in low- to middle- income countries (LMICs) where up to 85% of children under five have been exposed to *Campylobacter spp*. Nevertheless, there are gaps in understanding the infection sources, transmission routes, and specific genome lineages associated with Campylobacterosis outside high-income countries, where most national surveillance studies have been conducted. Inevitably, the global distribution of clonal complexes (CC) and sequence types (ST) remains largely uncharacterized. Characterizing the transmission and antimicrobial resistance (AMR) network trends in low-resource communities can impact intervention strategies and policymaking. This study aims to address this by analyzing a global collection of *Campylobacter* genomes from various sources to investigate species distribution and AMR trends. Using public databases (PubMLST, NCBI SRA), whole genome sequences were screened (≤ 500 contigs; genome size 1-2mbp), deduplicated, and analyzed for multi-locus sequence typing (MLST) and AMR genes via AMR Finder. Globally disseminated CC lineages such as ST21, ST353, and ST828, are associated with higher resistance, particularly within *C. coli*, where ST1150 is an emerging global threat. Regional variation highlighted unique lineages restricted to specific countries, for example ST362 in Peru and South Africa. The results emphasize necessity for focused interventions in LMICs, where specific clonal strains and resistance patterns present public health challenges. Identifying the global distribution and regional variation of strains can support public officials in developing tailored strategies and adjust antibiotic guidelines.

A293

Biofilm Alliance: A Network for Regulatory Sciences, Academic Research, and Industry Collaboration

Paulina Rakowska[1], James Redfern[2], Peter Askew[3], Gillian Irsdale[3], Geertje van Keulen[4]

[1]National Biofilms Innovation Centre, University of Southampton, Southampton, United Kingdom. [2]Department of Natural Science, Manchester Metropolitan University, Manchester, United Kingdom. [3]Industrial Microbiological Services Ltd, Hartley Wintney, United Kingdom. [4]Institute of Life Science, Swansea University Medical School, Swansea, United Kingdom

Abstract

Microbial biofilms significantly impact health, food safety, industrial processes, and the economy, costing an estimated US$5 trillion annually across various sectors such as food (US$91 billion), built environment (US$49 billion), and (waste-)water treatment (US$117 billion). Despite the scale of the scientific and economic opportunities and challenges related to biofilms, a critical absence of regulatory frameworks, standardised protocols and guidelines is blocking advances and real-world implementation of biofilm control technologies.

The Biofilm Alliance is a collaborative InnovateUK-funded Regulatory Science network bringing together academia, industry experts, metrology institutes, regulatory bodies, and standardisation agencies to bridge the gap between biofilm research and regulatory science.

Focussing on the built environment, industrial processes, food, and water sectors, the Alliance works to evaluate existing methodologies, recommend models, and create a structured framework for interpreting biofilm data with the ultimate aim of developing a collection of recommended Regulatory Science tools, supported by stakeholders' consensus.

Here, we will provide an update on the Alliance’s activities and achievements so far in data collection, gap analyses and engagements with regulators to identify shortcomings and guide interventions. Conference delegates will be informed about and invited to participate in ongoing Alliance activities and events including joining the Expertise Register and provide input for whitepapers and influencing of policy.

In summary, by fostering collaboration and supporting standardisation initiatives, the Biofilm Alliance will advance regulatory science related to biofilms to drive innovation, benefiting multiple industry sectors.

A294

Molecular detection and characterization of spoilage and opportunistic pathogen in commercially available milk in Northern Ireland

Mustasim Famous[1,2], John Neill[1], Karen Siu-Ting[1], Catherine Hambly[3], Linda Oyama[4]

[1]School of Biological Science, Queens University Belfast, Belfast, United Kingdom. [2]Department of Animal Science, Khulna Agricultural University, Khulna, Bangladesh. [3]School of Biological Science,Queens University of Aberdeen, Aberdeen, United Kingdom. [4]School of Biological Science,Queens University Belfast, Belfast, United Kingdom

Abstract

Milk is a highly nutritious and widely consumed food, therefore milk quality and safety are essential to prevent health risks, and for this reason, supermarket milk is pasteurised. Here, we assessed pasteurized milk from Northern Ireland supermarkets for microbial load, potential pathogens, and antimicrobial resistance (AMR) risks. Fifty milk samples were analysed using total viable counts (TVC) on Tryptic Soy and Nutrient Agar and total coliform counts (TC) on MacConkey Agar. Isolated colonies were identified through Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) and confirmed with 16S rRNA gene sequencing. AMR phenotypes were evaluated with MicroScan AST and compared to guidelines from the Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing.

Results showed that mean TVC and TC exceeded Commission Regulation (EU) No 365/2010 standards for pasteurised milk, with 20% of samples displaying TVC of 2.93 × 10^4 ± 1.3 × 10^2 CFU/mL and 10% showing TC of 1.3 × 10^2 ± 2.4 × 10^1 CFU/mL. The most prevalent species were *Pseudomonas spp., Acinetobacter spp., Streptococcus spp., Staphylococcus spp., Bacillus spp.,* and *Pantoea spp.,* some of which are known to cause opportunistic infections.

The presence of these bacteria, particularly those carrying AMR genes, indicates potential post-pasteurisation contamination, underscoring the need for stringent quality control measures in dairy production to safeguard milk safety and public health.

A295

Diversity and Transmission of *Campylobacter jejuni* in Wild and Domestic Avian Hosts

Mikolaj Marszalkowski, Oakem Kyne, Samuel Sheppard

University of Oxford, Oxford, United Kingdom

Abstract

Campylobacter jejuni is a leading cause of gastroenteritis in humans, as well as a common component of non-human animal microbiota. Consumption of infected chicken is the most common source of infection in higher income countries, leading to extensive research on *C. jejuni* in poultry. Though carriage has also been observed in wild birds, the diversity of *C. jejuni* within these hosts remains severely understudied. Understanding bacterial populations within wild reservoirs is important for preventing future disease outbreaks in humans. Furthermore, understanding transmission dynamics between wild and domestic birds is important for understanding anthropogenic effects on wild animal health and global *C. jejuni* ecology and evolution.

To understand patterns of bacterial adaptation and transmission between host species, we compare genome sequences of *C. jejuni* from different avian sources. All genomes in this study were freely obtained from PubMLST and were filtered for genome quality. This left genomes obtained from farmed chicken sources (n=17,613), other poultry (n=536), and wild birds (n=1,797).

In order to characterise the genomic diversity of *C. jejuni* found in avian hosts, the distribution of both MLST sequence types (STs) and clonal complexes is analysed to determine the most common STs and clonal complexes associated with different bird host species. To explore adaptation of *C. jejuni* to different bird species, we use Genome Wide Association Studies (GWAS) to search for specific loci associated with different hosts. Finally, analysis of phylogenetic trees generated from these genome sequences is used to infer patterns of *C. jejuni* transmission between different host species.

A296

Improving the prediction of antimicrobial resistance from genomic data for *Campylobacter*

Ava McCarthy Kerrigan, Martin Maiden, Samuel Sheppard, Frances Colles

University of Oxford, Oxford, United Kingdom

Abstract

Campylobacter species are the most common cause of foodborne bacterial gastroenteritis in the UK. Increasingly high levels of antimicrobial resistance globally are making severe infections much more challenging to treat. It remains unclear, however, how efflux pump genotypes across *Campylobacter jejuni* and *Campylobacter coli* lineages can predict resistance, complicating prevention efforts.

The CmeABC efflux pump is a key factor in multidrug resistance in these *Campylobacter* species, notably against ciprofloxacin and erythromycin, and actively expels antibiotics from the cell. Previous research has linked mutations in both the *cmeABC* promoter region and *cmeB* gene sequence to phenotypes of increased resistance. This project will investigate the association of various *cmeABC* alleles with resistance profiles, also examining whether co-occurrence of *cmeABC* mutations and established resistance determinants in gyrase A and 23S rRNA genes confer enhanced resistance.

There are currently over 86,000 *Campylobacter* spp. genomes in the PubMLST database. We have chosen 150 isolates from this, representing a wide range of clonal complexes to capture the existing phylogenetic diversity of the population. Each isolate will be subject to antimicrobial resistance testing and, following The European Committee on Antimicrobial Susceptibility Testing guidelines, we will determine minimum inhibitory concentration values to classify resistance levels. These phenotypic profiles will be compared with genotypic data, using respective genome sequences published on PubMLST.

Overall, this analysis will improve our understanding of how genotype predicts resistance phenotype in *Campylobacter*, and with results published in the PubMLST database, will provide valuable insights for the targeted treatment of challenging and persistent campylobacteriosis cases.

A297

Exploring the Efficacy of Omega-3 Fatty Acids as Antimicrobial and Antibiofilm Agents against *Streptococcus mutans*

Aoife Mulry ORCID iD

TUS: Midlands, Westmeath, Ireland

Abstract

Biofilms play a crucial role in development of oral diseases, particularly dental caries and are known to exhibit increased resistant to conventional treatments such as chlorhexidine.

This study evaluated antimicrobial and antibiofilm potential of Omega-3 fatty acids (FAs) against *Streptococcus mutans* ATCC 25175, a key pathogen in dental biofilm formation. The effects of FAs on *S. mutans* growth was determined at concentrations of 250, 100, 50, 25 and 10 µg/ml. Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) were determined, and metabolic activity was measured. Minimum Biofilm Inhibition Concentration (MBIC) was investigated using crystal violet staining and effects of artificial saliva on FAs was assessed. Assays measuring lactate dehydrogenase (LDH) and cell proliferation (XTT) were employed investigating cell toxicity and proliferation.

Results revealed that Eicosapentaenoic acid (EPA, C20:5), reduced bacterial growth at 10 µg/ml while Docosahexaenoic acid (DHA, C22:6) demonstrated potent antimicrobial effects, effectively inhibiting bacterial growth, reducing metabolic activity, preventing biofilm formation, and displayed log reduction value of 6 at 10 µg/ml. Notably, EPA and DHA antimicrobial activity was reduced to 25 µg/ml in the presence of artificial saliva. At 10 µg/ml both FAs showed lower cytotoxic compared to chlorhexidine. Furthermore, when incorporated into medical grade shellac coatings EPA and DHA effectively reduced biofilm formation on surfaces.

The findings of this study underscore the potential of Omega-3 fatty acids, particularly EPA and DHA as promising alternative antibiofilm agents against *S. mutans* offering an effective strategy for preventing dental caries while reducing cytotoxic effects.

A298

Repurposing of gut microbiome-derived peptides to treat infections caused by *Mycobacterium tuberculosis*

Gama Dominic ORCID iD[1], Samantha Collister[1], Rachel Wheatley[1], Suzie Hingley-Wilson[2], Ralf Bauer[3], Linda Oyama[1]

[1]Queens University, Belfast, United Kingdom. [2]University of Surrey, Surrey, United Kingdom. [3]University of Strathclyde, Glasgow, United Kingdom

Abstract

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is a global health concern that brought about 7.5 million new infections and 1.3 million deaths across the world in 2022. It is often termed the forgotten pandemic. New and alternative anti-TB regimens are of critical priority to increase the efficiency of the existing regimens, reduce the treatment duration, and to tackle the surge in antimicrobial resistance in Mtb strains. Here, we investigated the anti-TB activities of rumen microbiome-derived antimicrobial peptide (AMP) (15SecD) and novel chicken ceca microbiome-derived AMPs, Cecacins 9, 55, 56, 77, 94 and 103, against the double auxotrophic strain, Mtb H37Rv $mc^2$6206. We recorded potent activity with minimum inhibitory concentrations of 1-4 µg/mL via the resazurin microtitre assay. All peptides except cecacin 56, were indifferent with first-line drug isoniazid with a fractional inhibitory concentration index of 1-2. Peptides exhibited low haemolytic activity against human red blood cells and had high therapeutic indices ≥30, representing high antimicrobial specificity and promising therapeutic potential. The mode of action (MOA) of these peptides is under investigation using a variety of techniques, including confocal microscopy using membrane exclusion dyes. This has shown that AMP, 15 SecD, was able to permeabilise the Mtb cell membrane in comparison with cecacin-9, which had a minimal membrane activity and different MOA. Ongoing investigations into the molecular mechanisms of action of these peptides will provide insight about their treatment efficiency for multi-drug-resistant cases. Overall, we demonstrate the potential of gut microbiome-derived AMPs as treatment alternatives/or adjuvants for TB.

A299

Identification and characterisation of a novel serine protease produced by clinical isolates of *Achromobacter xylosoxidans*

Matthew McAteer[1], Katie Brown[1], Thomas Thompson[1], Tim Brown[2], Stephen Kelly[1], Brendan Gilmore[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]Renal Unit, Belfast Health and Social Care Trust, Belfast, United Kingdom

Abstract

Members of the genus *Achromobacter* have been increasingly implicated in a range of infections where they present as opportunistic pathogens, particularly in patients with cystic fibrosis (CF) or impaired immune systems. *Achromobacter* spp. exhibit an array of both intrinsic and acquired antimicrobial resistance mechanisms making infections caused by these organisms a challenge to treat. *Achromobacter xylosoxidans* is a recognised opportunistic pathogen in CF and whilst several virulence factors have been identified, their precise roles in pathogenicity are poorly understood. In this study we report the discovery of a novel extracellular, serine protease characterised from clinical isolates of *A. xylosoxidans*. Proteolytic activity was confirmed against a panel of fluorogenic substrates and protease mechanistic class was determined using class-specific inhibitors. The protease was effectively inhibited by PMSF and leupeptin, but not E-64 or EDTA, confirming the presence of an extracellular serine protease in *A. xylosoxidans* supernatants. Kinetic evaluation of several specific inhibitors was conducted and substrate specificity determined. Serine proteases are evolutionarily conserved across all domains of life, and their function in bacteria ranges from 'house-keeping' enzymes to critical virulence factors, to mediating processes including nutrient acquisition and invasion, and the evasion of the host's immune response. This study will characterise this novel enzyme, confirmed by LC-MS/MS fingerprint analysis and whole genome sequencing, as an important virulence factor of *A. xylosoxidans in vitro* and in an *in vivo* insect model of infection, thus contributing to the current understanding of *A. xylosoxidans* virulence.

A300

Analysing Life Identification Number codes to understand the effect of vaccine strategies on Neisseria meningitidis population structure

Faye Song ORCID iD, Kasia Parfitt, Keith Jolley, Odile Harrison, Ian Feavers, Martin Maiden

University of Oxford, Oxford, United Kingdom

Abstract

Invasive Meningococcal Disease (IMD) is a persistent and devastating global health issue caused by a vaccine-preventable pathogen, *Neisseria meningitidis*. Understanding the effects of meningococcal vaccines on *N. meningitidis* population structure is important for informing vaccine strategies. The novel Life Identification Number (LIN) code taxonomy for *N. meningitidis* offers a stable and high-resolution framework for genomic epidemiology. It has potential to reveal detailed patterns in genetic diversity and clonal structure.

This study leverages a curated dataset of IMD isolates from PubMLST. It spans epidemiological years 2010/11 to 2018/19, encompassing eight European countries with varying vaccination strategies: United Kingdom (n=1557), Czech Republic (n=27), France (n=254), Germany (n=272), Ireland (n=130), Poland (n=18), Spain (n=136), and Sweden (n=53). The differences in immunisation programmes provide a unique opportunity to assess how *N.* meningitidis population structure changes in response to vaccination. Openly accessible LIN codes for these isolates have been assigned within PubMLST.

The population structure across the period and between countries will be assessed using the LIN codes to infer the impact of vaccines. Our work will focus on the robust UK dataset to study trends in genomic epidemiology pre- and post-pandemic. Maximum Likelihood Trees, created with FastTree and adjusted for recombination with ClonalFrameML, provide an in-depth analysis through displaying LIN codes against genotypes in a multiple-ring structure.

Overall, this work investigates the power of LIN codes in revealing fine-scale genomic patterns, offering insights into how this system can be used to enhance our understanding of population biology and vaccination strategies.

A301

Chemical and Biological Assessment on Puerto Rican Native Plants from the Polygonaceae Family.

Karina Vega-Escobar, Pedro Sanchez-Hernandez, Ileana Rodriguez-Velez

University of Puerto Rico, Humacao, Puerto Rico

Abstract

The *Coccoloba* genus this group of plants is especially interesting as there are reports on the ethnobotanical uses. The Coccoloba uvifera have biological active compounds with antioxidant and antitumor activities that have been identified as flavonoids, tannins and terpenes. In consequence, we conducted the Antimicrobial Discovery kit results indicate a significant bacterial growth inhibition against mouth bacterias. Considering these preliminary results, we can hypothesize that these plants may contain potentially interesting secondary metabolites with biological activities. To evaluate their potential, we prepared ethanolic and chloroform-methanol (1:1) extracts of different parts of the plants under study and will perform the well diffusion assay to measure antimicrobial activity of the extracts against 5 Gram - bacteria. Also, we will conduct the Brine Shrimp Lethality test (BSLT) to evaluate the cytotoxicity of the extracts and the DPPH assay to determine their antioxidant properties. The long term purpose of this project is to identify the active compounds present in our crude extracts. Analysis of these mixtures will be performed HPLC and ^{1}H and ^{13}C NMR experiments. The result that we have conduct so fair have shown a preliminary activity in the DPPH assay in flower with a range of 40ug/ml-552ug/ml and in young leave was a range of 40ug/ml-640ug/ml. Also, we preformed the BSLT, and the results have been negative for the methanol/chloroform extract of the seeds, flower, green leaves and in the ethanolic extract of the young leave, and flower didn't show activity neither.

A302

Using a Life-Identification Number coding system to improve knowledge of Campylobacter transmission.

Kasia Parfitt, Keith Jolley, James Bray, Samuel Sheppard, Martin Maiden, Frances Colles

Oxford University, Oxford, United Kingdom

Abstract

Cases of human Campylobacteriosis, many resistant to antimicrobials, continue unabated worldwide, and more still needs to be done to elucidate routes of transmission. Molecular typing systems using multilocus sequence typing (MLST) have improved knowledge of host association by the organism, but core genome MLST (cgMLST) nomenclature used for fine typing is unstable. Life Identification Numbers (LIN codes) overcome this issue and are compatible with existing nomenclature.

A LIN code for *Campylobacter jejuni*, which causes 90% of human disease, was developed using the cgMLST v2 scheme. A subset of 1,739 isolates was randomly selected from 53,492 high quality *C. jejuni* genomes (<200 contigs, good cgMLST annotation) from the PubMLST *Campylobacter* database (https://pubmlst.org). A distance matrix was generated using the PubMLST Genome Comparator tool, from which dissimilarity thresholds were calculated. Silhouette (consistency) and Wallace (stability) coefficients were calculated using the MSTclust tool for each of the thresholds, and the adjusted Rand index used to assess compatability between thresholds and existing nomenclature, including sequence type and clonal complex.

The LIN code was validated using isolates from two water and dairy related outbreaks, and a longitudinal collection of human disease isolates from Oxfordshire. Results from these isolate collections, together with fine-type analysis of antimicrobial resistant lineages will be presented.

Session : Education and Outreach Symposium

A303

Making it relevant, engaging, and accessible: My experience adapting massive biofilm simulations into key stage 3-4 activities aligned to the national curriculum.

Joseph Weaver ORCID iD

Newcastle University, Newcastle, United Kingdom

Abstract

Creating educational material for key stages 3-4 (year 8 and above) as outreach is an excellent way to broaden our research impact. Although many researchers may feel that their work is not relevant at those levels, this is not the case. My work incorporates massive agent-based biofilm models and a specific competition mechanism – not traditional topics for a year 8 biology class. However, I have created an in-class activity for exactly that level.

The activity is a stand-alone web application (no online or server access required) which alternates between small instructional units, interacting with the results of pre-generated simulations, and semi-interactive formative assessments involving both pre- and post-instructional 'entrance ticket' and 'test your understanding' style questions.

The national curriculum was used to identify connections between research activities and concrete learning outcomes attached to specific lesson components. For example, a key stage 3 criterion under '*working scientifically*' asks that students "*make predictions using scientific knowledge and understanding.*" From that, specific learning objectives were developed, such as "the student will be able to explain what they believe will occur if the initial population ratio is changed, and why". That objective directly informed the creation of an interactive chart based on pre-generated simulations and associated assessments.

Microbial work grounded in solid research practices has core elements which align well with many of the items in the national curriculum. By using the curriculum to 'work with the end in mind' and consulting with practicing educators, we can greatly increase our impact.

A304

Learner-Based Strategies for Effective Teaching of Virus Structure to Beginners in Large Classrooms

Zubaida Hassan

Modibbo Adama University, Yola, Nigeria

Abstract

Teaching virus structures to beginners in large classroom settings presents unique challenges due to the difficulty in visualising viruses. Thus, resulting in passive learning with limited students' engagement, comprehension, and retention. In developing nations, these challenges are exacerbated by further infrastructural limitations. This abstract outlines an effective learner-based approach for teaching virus structure in a large classroom. After a brief introduction to virus and viral diversity and classification in a lecture format, the class was divided into small groups, each assigned to work on a virus. The work was to make a 3D model that shows the internal and external contents of the virus and present a poster that describes the virus structure, classification, and pathogenesis. The students used several materials including, mould, clay, plastics, foams, papers, paints etc. to build their models. Most of the groups make the models as a structure with two parts and arranged the internal contents on one if the halves. Once the two halves are closed, the internal contents are hidden, only the external features are seen as it is in the actual virus particle. The presentation makes the students search wide about their virus. This wide self-study exposed them to many similar viruses, and they understood the similarities and differences among the viruses. Thereby enhancing long-term learning. In conclusion, integrating interactive lectures, formative assessments, and peer-based activities that stimulate students' creativity is an effective approach to convey complex concepts and support students' understanding and retention even in large classrooms.

A305

Educating schools and the public about antibiotics and AMR; led by the Science, Art and Writing Trust.

Matt Hutchings ORCID iD, Jenni Rant

John Innes Centre, NORWICH, United Kingdom

Abstract

The Science, Art and Writing (SAW) Trust (https://sawtrust.org/) is a unique charity that brings science to life in the classroom using activities that combine science, art and writing. Typically, SAW workshops involve scientists, artists and writers taking over a class for the day, with engaging hands on science experiments that are followed by pupils interpreting the science through poetry, prose and art. SAW is based on the Norwich Research Park which is home to more than 100 microbiology groups that are all brought together under the virtual Centre for Microbial Interactions (https://www.cmi-norwich.ac.uk/) and a major research focus of the CMI is on Antimicrobial Resistance (AMR). This includes the development of rapid diagnostics, tracking the environmental spread of AMR genes, understanding plasmid-borne resistance and developing new antibiotics, including natural products and phage therapy. Increasing public awareness is also key to combatting AMR and the SAW Trust designed and delivered a programme of events and workshops with AMR researchers from across the NRP which culminated in the publication of a SAW Trust book entitled *Antibiotics*. This wonderful book documented these engagement activities and includes poems and artworks made by pupils in the school workshops. Here, we will share copies of the SAW book on Antibiotics and talk about the SAW Trust programme of outreach and engagement around AMR and the value of engaging with the public and school children through this unique combination of arts and science.

A306

Implementing virtual laboratory simulations to enhance student engagement and confidence: a comparative study between undergraduate and postgraduate cohorts

Chris Delaney, Leighann Sherry, Jason Brown

University of Glasgow, Glasgow, United Kingdom

Abstract

In recent years, there has been an uptake in the use of virtual laboratory simulations (VLS) relating to various scientific disciplines within education. These VLS provided essential training for students in key laboratory skills, that mimic real-world practicals, which were not possible during the Covid-19 pandemic. Following a return to in-person teaching, VLS continues to supplement learning alongside traditional on campus laboratories.

The purpose of this study was to compare student engagement and confidence in face-to-face laboratories, following access to a pre-session VLS. This platform provides a digital learning environment containing animated laboratory demonstrations and simulations for a range of common biological techniques. Students enrolled on the undergraduate Microbiology and MSc Oral Sciences programmes, were invited to take part in this collaborative study. Student engagement and confidence in the laboratory environment was assessed using quantitative questionnaires, pre- and post-practical sessions. In addition, qualitative data was collected using emotion coding, whereby emotions were recorded when observed in, or recalled by the participants. A control group containing students that did not receive the VLS prior to the laboratory sessions was used for comparison.

This project transcends different disciplines within the field of biomedical science and provides further opportunities for collaborative teaching practices within the broader field. Our findings will help guide future curricula design on whether VLS material should be utilised alongside traditional laboratory teaching methodologies, within this post-pandemic era.

A307

Meet the Viruses: Extended Reality for Microbiology Communication and Education

Rachael Suétt[1], Daniel Livingstone ORCID iD[1], Edward Hutchinson ORCID iD[2]

[1]The Glasgow School of Art, Glasgow, United Kingdom. [2]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

Despite the direct relevance of microbiology to people's lives, painfully highlighted by the COVID pandemic, misinformation and misconceptions about microbes are widespread and most people struggle to form a clear mental image of the microbial world. This is a particular challenge in secondary school education, where there are few resources to help students relate imaginatively to microbes and understand how microbes form part of the visible world. To help young and lay audiences engage with the science of viruses, and to encourage an interest in virology as a discipline, we created two extended reality tools which allow models of virus particles and their molecular components to be examined in 3D. The first app, 'Visible Viruses,' uses augmented reality to edit the view seen through a phone or tablet's camera, introducing visible virus particles into the users' environment. After exploring these models, and reading accompanying text that challenges common misconceptions about viruses and vaccines, users can test their knowledge through a quiz. The second app, 'Meet the Viruses,' is a virtual gallery displaying virus particles and proteins, which can be explored in-browser or by using a virtual reality headset. Both resources are freely available and are accompanied by lesson plans and printed resources. User testing demonstrates that they can be used with a wide variety of audiences, both children and adults. These flexible teaching tools help users develop a mental image of viruses as being part of the natural world: comprehensible, manageable and, often, beautiful.

A308

Building a sustainable future: how to redesign and reduce waste in the microbiology curriculum

Lucy Hunter, Loraine McDonald, Julie Alexander, Ellen Brown, Hazel Bracken, Lorraine Clark, Leighann Sherry ORCID iD

University of Glasgow, Glasgow, United Kingdom

Abstract

The world is in a state of climate crisis. Each year, 350 million tonnes of plastic waste is produced worldwide, with scientific research contributing 2% of this. One laboratory can produce >1 tonne of plastic waste/year, where the majority of this going to landfill and taking hundreds of years to degrade. Therefore, immediate action must be taken to reduce the waste produced by scientific laboratories.

This project aimed to implement sustainable practices across microbiology teaching laboratories at the University of Glasgow. Staff worked collaboratively with a final year student to evaluate undergraduate practices, before redesigning the curriculum to make it more sustainable.

Several adaptations were developed and implemented into the curriculum, with one practical session reducing the number of agar plates from 1,150 to 250 – a 78% reduction of single use plastics. This change led to no compromise in scientific outcomes or learning experiences, with only cost and volume of waste reduced. In addition, a new recycling stream for uncontaminated laboratory plastics was proposed to reduce unnecessary autoclave use and ultimately limit plastics sent to landfill. Moreover, water systems were protected by collecting all run off from staining materials for chemical waste, rather than being disposed down the sink and entering the environment.

Despite these successes, more can be done to further improve the sustainability of teaching and research laboratories. Despite this project being based in a microbiology laboratory, these measures can be adopted across all scientific disciplines to facilitate meaningful change and more sustainable practice across higher education.

A309

An update on microbiology education to secondary school biology teachers

Cheryl Walter[1], Snehal Kadam[1], Thiru Vanniasinkam[2], Charlotte Roughton[3]

[1]Hull York Medical School, Hull, United Kingdom. [2]Charles Sturt University, Bathurst, Australia. [3]Newcastle University, Newcastle, United Kingdom

Abstract

Microbiology outreach and education activities for school children are often well received. These activities, especially those delivered to secondary school children, aren't always linked to the AQA biology curriculum content, which contains microbiology theory and practical components. Here we briefly describe an online outreach activity prepared for 14 heads of secondary school biology teachers all associated with the same independent school group, United Learning. This group requested support from the Microbiology Society for new practical ideas in microbiology, and support in the methods of delivery of these practicals.

We delivered two sessions to the group of biology heads. One session highlighted what modern microbiology looks like and why it remains so relevant. The second session delivered a demonstration of key skills the group had identified they were less confident in, including streaking out onto solid media and colony forming units. This session also included ideas on how to augment the learning experience with the limitations most A level biology teachers have with microbiology practicals i.e., time, equipment and enhanced risk assessments. Interestingly a survey of this group of teachers, revealed that none of them received enough microbiology outreach in their own schools, only 14% had ever studied some form of microbiology themselves, and most would value more input into the microbiology curriculum.

This exercise might better inform future school outreach and education activities delivered by microbiologists working in higher education and help to align them more with the biology curriculum, while also informing on first year university microbiology curriculum design.

A310

Immersive tools for active and creative engagement on Bacteriophages

Maisie Czernuszka[1,2], Andrew Martin[1], George Dodgson[1], Paul Miller[3], Jo Fothergill ORCID iD[4], Ian Goodhead ORCID iD[1], Heather Allison ORCID iD[4], Chloe James ORCID iD[1]

[1]School of Science, Engineering and Environment, The University of Salford, Salford, United Kingdom. [2]Manchester Institute of Biotechnology, Manchester, United Kingdom. [3]East Street Arts, Patrick Studios, Leeds, United Kingdom. [4]Institute of Infection, Veterinary and Ecological Sciences (IVES), The University of Liverpool, Liverpool, United Kingdom

Abstract

The UK parliamentary inquiry into bacteriophages recognised their revolutionary potential to tackle antimicrobial resistance. Multiple barriers to the successful progression of phage therapy were also highlighted, including a need for wider societal understanding. We developed a set of working models of giant phage and bacteria as a simple and engaging way to demonstrate the different ways that phages affect bacteria. We also created a virtual reality (VR) lung experience to provide a real-world context and highlight some of the key challenges in phage therapy development. This study assessed the educational value of interactive phage infection games for inspiring public interest.

The utility of the phage infection activities was trialled at eight large public events including museums, summer schools, science festivals and art exhibitions, reaching more than 32,000 people between 2022-24. Informal feedback was overwhelmingly positive. A targeted survey during British science week revealed that 95% of public respondents found the models easy to use, with 100% reporting they learned something new. 90% understood specific mechanisms of phage infection and 76% were more likely to investigate microbiology further.

We adapted the interactive models to be entirely 3D-printable and created an open access digital DIY kit with instructions and learning resources so that anyone can run their own phage workshops. In this way we aim to broaden the reach and impact of phage research, promoting deeper and wider learning across a range of sectors to accelerate the progression of phage-based therapeutics and inspire the next generation of scientists to tackle global challenges.

A311

Drag Performance as a Methodology for Exploring Human-Microbe Relations: A Case Study of *Vibrio cholerae* and *Escherichia coli*

Natalie Beveridge ORCID iD[1], Rebekah Penrice-Randal ORCID iD[2], Hwa Young Jung[3], Rachel Seoighe[4], Trude Sundberg ORCID iD[5], Annalaura Alifuoco ORCID iD[6]

[1]Mersey and West Lancashire Teaching Hospitals NHS Trust, Prescot, United Kingdom. [2]Center for Proteome Research, University of Liverpool, Liverpool, United Kingdom. [3]Independent Artist, Liverpool, United Kingdom. [4]Hearth Consultancy, Margate, United Kingdom. [5]School of Social Sciences, University of Kent, Canterbury, United Kingdom. [6]School of Creative and Performing Arts, Liverpool Hope University, Liverpool, United Kingdom

Abstract

Background

Traditional science communication often relies on unidirectional knowledge transfer, limiting deeper engagement with microbial worlds. Our project employs drag performance art to disrupt this paradigm and explore the complex entanglements between human and microbial histories.

Methods

Through a transdisciplinary collaboration between microbiologists, sociologists, ecologists, historians, and artists, we investigate human-microbe relationships through the lens of water-borne bacteria. Community workshops in Liverpool and Margate examine historical cholera outbreaks and contemporary sewage pollution, using drag performance to embody and interpret the perspectives of *Vibrio cholerae* and *Escherichia coli*. This methodology enables exploration of challenging microbial subject matter while providing creative outlets for processing public health concerns. Participatory workshops will complete in February 2025.

Preliminary results

Drag as a disruptive methodology in science communication enables:

1. Reframing human-microbe relationships beyond pathogen-host binaries
2. Exploration of microbial agency in shaping human history / public health
3. Generation of community-led narratives about local water ecologies
4. Challenges the traditional hierarchies in scientific knowledge production

Conclusions

By queering conventional science communication through drag performance, we create novel spaces for understanding microbial-human entanglements. This approach generates new insights into how communities conceptualize and relate to microorganisms, while advancing methodological innovations

in public engagement with microbiology. Workshop outputs will inform development of educational materials for broader public dissemination in Summer 2025.

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Session: Champions of Change: Celebrating Actions Advancing Equality, Diversity, and Inclusion

A313

Discussions on Neurodiversity and Promoting Support and Inclusion in Microbiology Research

Cailean Carter[1], Charlotte Roughton[2], Kirsty Jones[3], Becky Thomas[4], Kevin Maringer[5], Sara Henderson[6]

[1]Quadram Institute, Norwich, United Kingdom. [2]Newcastle University, Newcastle, United Kingdom. [3]Staffordshire University, Stoke-on-Trent, United Kingdom. [4]School of veterinary medicine, Surry University, Guildford, United Kingdom. [5]The Pirbright Institute, Pirbright, United Kingdom. [6]Centre for Skin Sciences, University of Bradford, Bradford, United Kingdom

Abstract

We intend to present a poster aimed at priming discussions on neurodiversity and promoting support and inclusion in microbiology research. Neurodiversity describes the neurocognitive differences between people, emphasising the different ways in which people perceive and think. Neurodivergence is an umbrella term used to describe those outside the cognitive norm and are often associated with Attention Deficit Hyperactivity Disorder, Autism Spectrum Condition, dyslexia, and many other conditions. Despite those who are neurodivergent providing unique ways of thinking that greatly benefit microbiology research, it is legally considered a disability and comes with extra challenges in progressing through academia. Despite a quarter of the UK population having a disability, only 11% of BBSRC-funded students, 8% of UKRI funded postdocs, and 2% of principle and co-investigators awarded UKRI grants have disclosed a disability (according the UKRI Equality, Diversity & Inclusivity data). There are evident challenges with career progression in academia for neurodivergent students and staff. As such, we want to use this poster as a discussion point for attendees to share their experiences, provide feedback, and build support networks that can help shape a more accessible microbiology research environment. We will use these discussions to feed back into actionable points the Microbiology Society can use to promote accessibility at their events, accessing funds, and publishing

A314

Empowering Women in Microbiology: Actionable Strategies for Global Equity

Sharon M Brookes ORCID iD[1,2], Molly Cohen[2], Shara Cohen[2]

[1]University of Surrey, Guildford, United Kingdom. [2]Mum's in Science, High Barnet, United Kingdom

Abstract

Globally, women make up only 28% of the workforce in STEM (science, technology, engineering, and mathematics), and in microbiology, the gender gap is particularly pronounced in leadership roles, with women holding less than 20% of senior positions in academia, non-academic institutions and industry. This poster highlights key issues faced by women in microbiology, including gender biases, pay inequality, lack of mentorship and role models, and limited access to leadership opportunities. We will present insights into these barriers and offer actionable strategies to achieve equity. Our new Women in STEM platform is designed to address these challenges by providing a global networking solution. By offering peer support, tailored resources, and industry connections, this platform is set to make a significant impact on the inclusion of women in STEM and microbiology specifically. This platform will provide microbiologists with practical tools to support equality, inclusion and gender diversity, in their own environments.

BLOCK B

Session : Virus Forum

B001

Developing a bivalent vaccine to reduce the risk of Nipah virus outbreaks in pigs

Crystal Ng[1], Jack Hayes[1], Walter Fuchs[2], Debbie Noel[1], Andrew Bentham[3], Aswin Doekhie[4], Anne Balkema-Buschmann[2], Asel Sartbaeva[4], Alison Wakeham[3], Michael Francis[5], Barbara Klupp[2], Simon Graham[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]Friedrich-Loeffler-Institut, Rübenberge, Germany. [3]Global Access Diagnostics, Thurleigh, United Kingdom. [4]Ensilitech, Bristol, United Kingdom. [5]BioVacc Consulting, Amersham, United Kingdom

Abstract

Bat-to-pig-to-human transmission caused the first and still most severe Nipah virus (NiV) outbreak in Malaysia and Singapore, leading to the culling of almost half the Malaysian pig population and significant economic damage. Despite the threat NiV poses to pig dense countries of Southeast Asia, no licensed vaccines exist, and the rare nature of outbreaks limits commercial interest. Our project aims to address this gap by developing a bivalent vaccine that would protect pigs against infection from a prevalent virus i.e., the pseudorabies virus (PrV), as well as NiV. Immunogenicity trials with a first-generation vaccine candidate using the PrV Bartha K61 strain engineered to express soluble NiV prefusion stabilized (pre)F and G glycoproteins showed immune responses to NiV, without affecting PrV-specific responses. However, the data suggested that two doses may be needed to provide protection against NiV. To improve immunogenicity, and potentially provide efficacy with a single shot, recombinant PrV Bartha K61 expressing full-length, membrane displayed, NiV preF and G was constructed. The data from an ongoing immunogenicity trial comparing the immunogenicity of the first- and second-generation constructs in pigs will be presented. In preparation for the vaccine candidate potentially entering commercial development, the results from an evaluation of the propagation of the recombinant PrV vaccine candidate in suspension cell cultures will additionally be presented.

B002

The Hunt for Maedi Visna Resistance: Understanding the Current TMEM154 Genetic Situation within the UK National Flock.

Scott Jones ORCID iD[1], Fiona Lovatt[1,2], Samuel Boon[3], Samir Id-Lahoucine[4], Kevin Mclean[5], David Griffiths[5], Rachael Tarlinton[1]

[1]University of Nottingham, Nottingham, United Kingdom. [2]Flock Health Ltd, Barnard Castle, United Kingdom. [3]AHDB, Coventry, United Kingdom. [4]SRUC, Edinburgh, United Kingdom. [5]Moredun, Penicuik, United Kingdom

Abstract

Maedi Visna (MV) is a chronic lentiviral disease of sheep which currently affects just under 10% of the UK national flock. The disease has a long latency period ranging from months to years before being detectable by diagnostic methods or showing clinical signs and ultimately ends in fatality. These factors in conjunction with no available treatment or vaccination makes controlling viral spread difficult.

Current control strategies rely on a voluntary programme of routine testing and culling of infected animals, which can be costly to farmers and has limited efficacy at a national level. An alternative option going forward is selective breeding for traits showing genetic resistance to viral infection. Multiple large genome-wide association studies and targeted field studies have highlighted a mutation in Transmembrane protein 154 (TMEM154), an E to K amino acid change at position 35, to be strongly associated with decreased risk of MV.

Before implementation of such a strategy, there are some questions that need to be addressed. Firstly, what does the TMEM154 protein do during infection and would selection for the mutation result in deleterious phenotypes. Secondly, what is the current prevalence of the mutation within the UK flock as a whole and within individual breeds. The second question is being addressed in this current work.

Our approach takes animals from the most utilised breeds within the current UK stratified sheep production system to be genotyped. Allele frequency is assessed, allowing us to form a current snapshot of TMEM154 genetics with the UK.

B003

Hepatitis B Immunity Status Among Healthcare Personnel at Thammasat University Hospital, Thailand

Wanwarang Hiriote, Siripen Tor-Udom, Pharuhat Tor-Udom, Nuntra Suwantarat

Thammasat University, Pathumthani, Thailand

Abstract

Background: Hepatitis B virus (HBV) infection in healthcare personnel (HCP) is major concern due to the high risk of exposure to blood and body fluids from infected patients. This study aimed to determine the level of protection against HBV infection among HCP at Thammasat University Hospital.

Methods: Hepatitis B surface antibody (anti-HBs) titers were tested in a total of 612 blood samples from HCP using Chemiluminescence immunoassay. A correlation between Hepatitis B vaccination and several factors was analyzed using logistic regression. A comparison between two groups who completed vaccination within 5 years (Group I) and more than 5 years (Group II) was performed using the Chi-square test.

Results: Of the 612 HCP included, 79.9% were vaccinated, and 20.1% were unvaccinated. The logistic regression analysis indicated that age, marital status, gender, education level, and occupation were significantly associated with vaccination. Among the vaccinated HCP, 7.64% had an anti-HBs titer <10 mIU/mL, 23.35% had a titer between 10-100 mIU/mL, and 69% had a titer >100 mIU/mL. The trend in anti-HBs titers decreased as the time since vaccination increased, from 460 mIU/mL in Group I to 270 mIU/mL in Group II.

Conclusion: Most HCP were vaccinated and had adequate antibody levels to protect against HBV infection. Nevertheless, antibody levels decline after 5 years of vaccination. Therefore, it is recommended to measure antibody levels, administer a booster dose, and screen for HBs antigen as a requirement for HCP.

Keywords: Healthcare personnel, Hepatitis B vaccine, Hepatitis B antibody

B004

Tick the Box for Immunity: Investigating Antiviral Mechanisms in *Rhipicephalus microplus* (BME/CTVM6) tick cells

Zimo He[1], Lesley Bell-Sakyi[2], Marine Petit[1]

[1]University of Surrey, Guildford, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Rhipicephalus microplus is the tick vector responsible for transmitting severe fever with thrombocytopenia syndrome virus (SFTSV), prevalent in East Asia. Despite the significance of SFTSV and other tick-borne viral diseases, our understanding of the immune responses in ticks, specifically their ability to recognise and combat viral infections, remains limited.

Our study investigates how BME/CTVM6 tick cells, derived from *R. microplus* embryos, respond to double-stranded RNA (dsRNA) detection. We used fluorescently labelled poly (I:C) as a viral dsRNA mimic to investigate if BME/CTVM6 cells internalise and detect dsRNA. Detection of poly(I:C) confirmed internalisation within BME/CTVM6 cells, with localisation near the nucleus 12 hours post-transfection, suggesting efficient release from endosomes. This suggested that the synthetic dsRNA was internalised and detected by our tick cells, however its action as an immunomodulator remains unknown. To test our hypothesis that introducing viral dsRNA can activate an antiviral response in ticks, we examined whether genes associated with antimicrobial function would be regulated following transfection. We selected known arthropod antiviral effectors and assessed the expression level by RT-qPCR. By comparing time-points and immune-stimulation level we aim to depict poly(I:C) function in *R. microplus*.

Our work shows for the first time that tick cells can detect viral dsRNA upon infection. It also provides the first screening of antiviral peptide activity in tick cells, opens avenues for future characterisation of innate immune pathways in *R. microplus* and, by enhancing our knowledge of tick immunology, is crucial for developing effective control strategies for tick-borne diseases.

B005

Complement-dependent cytotoxicity against African Swine Fever Virus

Sian Wells ORCID iD[1,2], Basu Paudyal[1], Lynnette Goatley[1], Christine Rollier[2], Chris Netherton[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Surrey, Guildford, United Kingdom

Abstract

African swine fever virus (ASFV) is a large complex DNA virus that causes African swine fever, a haemorrhagic disease of pigs with up to 100% mortality. No commercially licensed vaccine or treatment is currently available. Antibody responses against ASFV have been shown to be protective, but neutralisation is technically challenging to demonstrate and its role in protection is controversial. Non-neutralising antibody functions such as complement-dependant cytotoxicity (CDC) have been demonstrated previously using 51Cr release assay, but the role they play in ASF infection is not well known. Here we show development of a novel CDC assay using a DNA intercalating dye and a fluorescent plate reader and confirm that it can detect CDC using an influenza HA-expressing cell line and anti-HA antibody. We also demonstrate that this assay can detect ASFV-specific CDC using sera from animals exposed to two different high virulence ASFV isolates. We then use this CDC assay to compare the presence of CDC-mediating antibodies with outcome of disease in experimentally vaccinated animals. This will contribute to the understanding of the B cell responses to ASFV and ASFV vaccines, protective or detrimental, which may aid future vaccine development.

B006

The role of African Swine Fever core shell proteins during the early stages of replication.

Ashleigh Tungate[1,2], Lynnette Goatley[1], Gill Elliott[2], Christopher Netherton[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Surrey, Guildford, United Kingdom

Abstract

African swine fever (ASF) is a lethal haemorrhagic disease of domestic pigs and wild boar that causes mortality rates of up to 100%. Due to the lack of commercially licenced vaccines or treatments, control relies on strict biosecurity and culling of infected herds and therefore outbreaks of ASF result in high economic losses for the pig industry.

ASF is caused by a large dsDNA virus (ASFV) that has a genome of 170-190 kbp in size and virions contain at least 68 viral proteins. Virion cores enter the cytoplasm after fusion of the inner envelope with endosomal membranes, and 6 to 8 hours later viral factories begin to form in perinuclear regions of the cell where virus assembly takes place. Between entry and virus factory formation, a poorly understood nuclear stage of replication takes place in which short sections of viral DNA are synthesised prior to export to the cytoplasm. To gain a better understanding of this stage of the virus life cycle, the localisation of all core shell proteins, their interactions with the host cell and the functions these have during early ASFV infection will be assessed.

A comparative analysis of the localisation of viral core proteins in infected cells and when transiently overexpressed was undertaken. Five components of the core were present in the nucleus when overexpressed by transient transfection. The localisation of nuclear bodies, nuclear speckles and lamina was also analysed to assess the effect of both virus and individual core proteins on nuclear substructures.

B007

Optimising Murine Norovirus Density Gradient Centrifugation as a Purification Protocol for the use in Novel Molecular Studies

Abigail Shires ORCID iD, Jake Mills ORCID iD, Morgan Herod ORCID iD, Caroline Chilton ORCID iD

University of Leeds, Leeds, United Kingdom

Abstract

Human Norovirus (HuNoV) is a major human enteric pathogen that causes ~685 million cases of diarrhoea and ~200,000 mortalities worldwide per annum. Infection disproportionally affects those working in community-based healthcare settings, lower income countries, and the global south. Due to difficulties associated with culturing HuNoV, murine norovirus (MNV) is used as a safe, easily culturable and tractable model system for studying fundamental virus biology. Detailed molecular studies often require purified stocks of virus, thus allowing virions and their interactions to be elucidated under precisely controlled experiments. In order to separate virions from cell culture components, a series of purification steps such as density gradient centrifugation (DGC) can be performed.

Here, we use quantitative and qualitative techniques to investigate the loss of viable MNV during separation processes. Using a combination of electron microscopy, SDS-PAGE, western blotting, and infectious virus assays, we observed an up to ~95% decrease in infectious virus particles throughout DGC. Longitudinal stability studies suggested purifying virions significantly increased the stability of infectious particles by 4-logs after 6 months compared to unpurified controls. In conclusion, ultracentrifugation gradient separation techniques are an inexpensive way of separating virions from cellular component. Furthermore, using a series of purification steps that gradually increases the relative ratio of viral to host cell proteins, allows this technique to suit the specific requirements of individual downstream applications. Work is ongoing to incorporate and compare alternative purification methodologies.

B008

Origin and functional divergence of TRIM25 and Riplet

Syed Ibrahim Syed Mohamad Jamil[1], Sheng Chen[1], Jennifer Bohn[2], Daniel Goncalves-Carneiro ORCID iD[1]

[1]Imperial College London, London, United Kingdom. [2]The Rockefeller University, New York, USA

Abstract

TRIM25 and Riplet are essential components of responses to virus infections, sharing high sequence homology and domain topology. However, how their substrate specificity impacts nucleic acid sensing is not well understood. Here, we investigated the origin, evolution and functional divergence of these two proteins. Phylogenetic analyses revealed that TRIM25 and its paralogue Riplet share a common ancestral gene that emerged prior to the diversification of Osteichthyes, with the Riplet lineage diverging from TRIM25 in early vertebrates. We examined the interaction partners of TRIM25 and Riplet, identifying that TRIM25 preferentially binds to the zinc finger antiviral protein (ZAP), whereas Riplet specifically associates with RIG-I; these interactions are mediated through the SPRY domain in both proteins. Moreover, expression of TRIM25 leads to the destabilisation of ZAP but increases RIG-I stability. Conversely, Riplet negatively impacts RIG-I expression but does not impact ZAP. Structural analysis of TRIM25-ZAP complexes revealed that their interaction is stabilised by four salt bridges between the N-terminal domain of ZAP and the SPRY domain of TRIM25. Finally, we assessed the roles of TRIM25 and Riplet in nucleic acid sensing. Riplet was found to be essential for immune activation in response to short double-stranded RNA (dsRNA) but not poly I:C or CpG-rich RNA. In contrast, TRIM25 is critical for ZAP's antiviral function yet dispensable for the recognition of dsRNA or poly I:C. Together, our findings elucidate the evolutionary pathways and distinct immune functions of TRIM25 and Riplet, highlighting their specialised roles in vertebrate antiviral defence mechanisms.

B009

Characterising the host-pathogen interactions of henipaviruses

Jakub Hantabal[1], Cillian Gartlan[1], Luke Jones[1], Phalguni Rath[2], Bernadett Palyi[3], Zoltan Kis[3], Sophie Smither[4], Javier Salguero[5], Julian Hiscox[6], Rachel Ireland[4], Miles Carroll[1]

[1]Pandemic Sciences Institute and Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom. [2]Centre for Human Genetics, University of Oxford, Oxford, United Kingdom. [3]National Center for Public Health and Pharmacy, Budapest, Hungary. [4]Defence Science and Technology Laboratories, Salisbury, United Kingdom. [5]United Kingdom Health and Security Agency, Salisbury, United Kingdom. [6]Institute of Infection, Veterinary & Ecological Sciences, University of Liverpool, Liverpool, United Kingdom

Abstract

Nipah virus (NiV) and Hendra virus (HeV) are pathogens of international concern due to their high virulence and lack of effective vaccines and therapeutics, resulting in high mortality. Both NiV and HeV cause severe respiratory disease and encephalitis. The clinical progression of this disease is well understood; however, vaccine and therapeutic development is hindered by lack of knowledge surrounding the interactions between a henipavirus infection and the host cells, tissues and the immune system.

We aim to address this gap by studying the henipaviral infection in a series of *in vitro* and *in vivo* models.

NiV is known to infect deep cortical neurons in the brain, however, knowledge is missing on tropism towards other nervous system cell types. To characterise this, we will employ iPSC-derived organoid whole brain and cortical models. These will be infected with NiV and subjected to immunofluorescence imaging for NiV proteins and characterised with a diverse panel of neurologically relevant cells.

NiV is known to cause lesions in the infected brains and lungs. However, the composition of the immune infiltrate at the lesions is unknown. We will elucidate this by utilising the multiplex immunohistochemistry platform CellDIVE, on samples from infected Syrian hamster tissues challenged with low dose of NiV mimicking natural infection. This will be the first experiment conclusively establishing the composition of tissue immune infiltrate in a NiV infection.

In conclusion, this project will establish pioneering knowledge into the neurological tropism of NiV as well as immune infiltrate in brain and lung infection.

B010

Pathways to Persistence: Unravelling Orthonairovirus Infection Mechanisms in Tick and Mammalian Hosts.

Harry Taylor ORCID iD[1], Marine J. Petit[1], Lesley Bell-Sakyi[2], Sophie J. Smither[3]

[1]Department of Microbes, Infection and Immunity, School of Biosciences, FHMS, University of Surrey, Guildford, United Kingdom. [2]Department of Infection Biology and Microbiomes, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom. [3]CBR Division, Defence Science and Technology Laboratory (Dstl), Porton Down, Salisbury, United Kingdom

Abstract

Crimean-Congo haemorrhagic fever virus (CCHFV) is a tick-borne orthonairovirus causing severe hemorrhagic disease in humans, with a mortality rate up to 30%. Recent geographical expansion of CCHFV in Southern European countries where *Hyalomma* ticks are established raises major public health concerns, as no treatment or vaccines exist. Primarily transmitted through bites from infected *Hyalomma* ticks, understanding CCHFV infection in both host and vectors will inform efficient control strategies.

The ability of tick-borne orthonairoviruses to cycle between mammalian and tick hosts, combined with their limited number of proteins, suggests that CCHFV exploits common cellular pathways in both hosts to facilitate infection and replication. To identify and characterize these pathways, we are comparing infection in tick (*Hyalomma spp.*) and mammalian cell lines, initially focusing on the less virulent orthonairovirus Hazara (HAZV). We established methodologies and standardized viral kinetics, RNA replication, and protein localization analyses across both host taxa. Once validated with HAZV, these methods will be applied to CCHFV to compare infection dynamics in mammalian and tick cells.

In future studies, we will combine detailed characterization of orthonairovirus infections with high-throughput analyses to reveal both unique and shared mechanisms in mammalian and tick hosts. This approach aims to deepen our understanding of CCHFV biology and evaluate the suitability of HAZV as a model virus and could lead to the identification of new drug targets.

B011

Molecular mechanisms of coronavirus recombination

Joe Hemsley-Taylor ORCID iD[1,2], Nuno Brito Santos ORCID iD[1], Ciarán Gilbride ORCID iD[1], David Bauer ORCID iD[1]

[1]The Francis Crick Institute, London, United Kingdom. [2]University of Bath, Bath, United Kingdom

Abstract

Recombination contributes to the evolution and diversification of coronaviruses and is a causative agent in viral host switching event, as well as driving antigenic changes within viruses circulating in humans. Over the past three years, in SARS-CoV-2, recombination between omicron sublineages has led to the emergence of new variants which contribute towards antigenic escape. However, the molecular mechanism of recombination remains unknown.

We studied recombination in the seasonal human coronavirus HCoV-OC43, the most closely related betacoronavirus to SARS-CoV-2 that circulates the human population. Using an in-house reverse genetics system, we developed an *in vitro* assay which enabled us to study the effects of viral and host factors on recombination frequency. We designed genomic spike deficient viral particles packaged with host cell-derived spike protein. These single round infectious particles allow us to select for the acquisition of viral RNA within recipient host cells back into the deficient viral genome to generate infectious virus.

By isolating and sequencing recombinant progeny, we can detect recombination hotspots over the viral genome and determine the likelihood of certain recombination events. Therefore, these results will help predict the probability of zoonotic spillover and immune evasion. As a result, our study will provide a greater understanding into the frequency and molecular workings of recombination and therefore coronavirus evolution.

B012

RSV activation of Wnt/β-catenin signalling in airway epithelial cells is dependent on epithelial-mesenchymal crosstalk

Telma Sancheira Freitas, Carlos Maluquer de Motes, Lindsay Broadbent ORCID iD

University of Surrey, Guildford, United Kingdom

Abstract

Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infection (LRTI) in young children. LRTIs during early childhood have been associated with decreased lung function in later life and an increased risk of premature death from respiratory disease. However, the role of RSV in the development and/or progression of chronic lung disease (CLD) is not fully understood.

There is extensive evidence that dysregulated Wnt/β-catenin signalling contributes to CLD, but its regulation during RSV infection is unknown. We hypothesised that epithelial-mesenchymal cell crosstalk was required for Wnt/β-catenin signalling during RSV infection, and that this contributes to CLD pathogenesis. Using a well-differentiated airway epithelial cell/fibroblast co-culture model (WD-PAEC/MRC5), we showed that, at 24-hours post infection (hpi), RSV results in significant upregulation of Wnt signalling markers (*CTNNB1* and *AXIN2)* in airway epithelial cells of WD-PAEC/MRC5 co-cultures. This was not noted at later timepoints. No Wnt signalling was observed in RSV infected single WD-PAEC cultures. Furthermore, RSV infection of WD-PAEC/MRC5 co-cultures results in the upregulation of *RNF43*, a downstream target gene of Wnt/β-catenin that functions as a negative regulator of this signalling cascade, at 24hpi and 96hpi.

Our data suggests RSV-induced Wnt/β-catenin pathway activation in epithelial cells is dependent on the presence of fibroblasts. Following RSV infection, Wnt/β-catenin signalling is activated early in infection and dampened in late infection in co-culture models derived from healthy adults. Future work will determine if Wnt/β-catenin regulation is dysregulated in co-culture models derived from patients with CLD.

B013

Finding Host Journey for Pseudomonas aeruginosa LESB58 Prophage 5

REEM ALMERAN[1,2], Revathy Krishnamurthi[1,3], Enrique González-Tortuero[1,4,5], Chloe James[5], Jo Fothergill[1], Heather Allison[1]

[1]Institute of Infection, Veterinary and Ecological Sciences (IVES), Liverpool, United Kingdom. [2]College of Environmental sciences, University of Mosul, Mosul, Iraq. [3]Quadram Institute of Biosciences, Norwich, United Kingdom. [4]Faculty of Health and Life Sciences, Newcastle, United Kingdom. [5]School of Science, Engineering and Environment (SEE), Manchester, United Kingdom

Abstract

The Liverpool epidemic strain (LES) of *Pseudomonas aeruginosa* causes transmissible chronic respiratory infections that negatively impact the functions of the cystic fibrosis lung and decrease the effectiveness of antibiotic therapies.

Several prophages have been detected in the accessory genome of LES and suggested to play a role in the competitiveness of their host. Although the infective properties of LES phages ϕ2-4 have been characterised, LES ϕ5 is yet to be isolated. In order to experimentally determine the impacts of LES ϕ5 carriage, a host capable of being infected by LES ϕ5 is required. To begin the study of LES ϕ5 a thorough bioinformatic-based study was first performed. We determined the total length of the LESϕ5 prophage to be 50,235 bp, with the attL and attR regions located at 2,690,327 – 2,690,341 and 2,740,547 – 2,740,561 in the *P. aeruginosa* LESB58 genome, respectively. Additionally, we identified several new putative gene functions and proved that LES ϕ5 is a complete, inducible phage through detection of encapsidated LES ϕ5 DNA in induced and DNAse-treated LESB58 supernatants.

Plaque assay and spot assays were used in attempted to isolate infective LES ϕ5 from filtered supernatants of LESB58 under a range of culture conditions. Screening of 34 potential *P. aeruginosa* host strains yielded no clearing or plaques that could be attributed to LESϕ5 lysis. It was thus speculated that common phage defence systems may preventing productive infection. Work is ongoing to synthesise LESϕ5 on a conjugatable plasmid to ensure that LES ϕ5 is introduced into *P. aeruginosa*.

B014

Sequestration of ADAMTS4 by Respiratory Syncytial Virus Inclusion Bodies

Ruby Ryan[1], Fatoumatta Jobe[2], Danielle Debruin[1], Telma Sancheira Freitas[1], Salvatore Santamaria[1], Dalan Bailey[2], Lindsay Broadbent[1]

[1]University of Surrey, Guildford, United Kingdom. [2]Pirbright instituite, Pirbright, United Kingdom

Abstract

Replication and transcription of respiratory syncytial virus (RSV) occurs in membrane-less cytoplasmic condensates named inclusion bodies (IBs). This is mediated by the multivalent interactions between the viral N/P proteins. Reports have confirmed that IBs sequester immune proteins, dampening innate immune responses. We aimed to further elucidate the impact of innate immune protein sequestration on the immune response to RSV.

Confocal imaging was performed to determine if other important innate immune proteins are sequestered during RSV infection. The role and impact of this sequestration was explored through qPCR, viral titration, and protein quantification. Furthermore, we exploited the ability of the RSV N and P protein to form 'pseudo-IBs' to elucidate their role in immune modulation in an RSV independent experimental design.

Our research reveals that whilst other IB-forming viruses are able to sequester innate immune proteins such as STAT1/STAT2, RSV does not sequester these in airway epithelial cells. Our findings demonstrate for the first time that members of the ADAMTS family are sequestered in RSV IBs.

ADAMTS4, a proteolytic enzyme, contributes to tissue remodelling during respiratory viral infection, increasing immune cell infiltration. Following infection of human and bovine fibroblasts ADAMTS4 is sequestered in IBs. This suggests that this is a common phenomenon of orthopneumoviruses. Additionally, the absence of this sequestration observed in homologous proteins (ADAMTS1 and ADAMTS5) in epithelial cells, indicates a potential for fibroblast-specific sequestration of ADAMTS proteins. Further work is required to elucidate the mechanisms leading to this sequestration and to explore the consequences on its downstream targets.

B015

Entry of SARS-CoV-2 during cell-to-cell transmission, as opposed to cell-free infection, occurs independently of TMPRSS2 and evades humoral immunity

Saskia Stenzel ORCID iD, Christine Goffinet

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Abstract

Enveloped viruses spread via both cell-free virus particles and direct cell-cell contacts. Cell-to-cell transmission accelerates viral spread and promotes immune evasion by escaping neutralisation by human antibodies targeting the viral glycoprotein.

Here, we studied the efficiency of both transmission modes in the context of SARS-CoV-2 infection. Target cells infected with a GFP-expressing reporter virus for cell-free infection or cocultured with virus-producing donor cells for direct cell-to-cell transmission, shared similar percentages of GFP-positive cells and Remdesivir sensitivity. The cellular receptor ACE2 and the serine-protease TMPRSS2 are two key entry factors for SARS-CoV-2 infection. Both transmission modes were equally sensitive to neutralisation of ACE2 on target cells by monoclonal anti-ACE2 antibodies. However, inhibition of TMPRSS2 by camostat treatment only poorly prevented virus spread in cocultures, while effectively decreasing the rate of cell-free infection, suggesting an inferior role of TMPRSS2 during entry via cell-to-cell transmission. Furthermore, we investigated the sensitivity of both transmission modes to humoral immunity. Incubation with monoclonal antibodies targeting the SARS-CoV-2 spike receptor-binding domain or with sera from vaccinated and reconvalescent donors resulted in inhibition of infection by cell-free particles in a concentration-dependent manner, whereas cell-to-cell transmission was refractory to spike neutralization. Current work focuses on the response to cell-intrinsic immunity and kinetics of cell-free infection compared to cell-to-cell transmission.

Together, our data suggest that entry of SARS-CoV-2 in the context of cell-to-cell transmission is resistant to or antagonizes humoral immunity and differs in its cofactor requirement from cell-free infection.

B016

Deciphering the mechanism of attenuation of a novel avian coronavirus vaccine candidate

Amber Tappin, Katalin Foldes, Renata Fleith, Erica Bickerton, Sarah Keep, Trevor Sweeney

The Pirbright Institute, Woking, United Kingdom

Abstract

Infectious bronchitis virus (IBV) is a highly contagious *gammacoronavirus* infecting domestic fowl worldwide, causing significant economic loss in the poultry industry. IBV causes respiratory distress, rendering birds vulnerable to secondary infections, whilst also reducing egg production. Currently, live attenuated vaccines (LAVs) are predominantly used to control IBV, with little cross-protection between strains. LAVs are generated via serial passage in embryonated hens' eggs with the exact mechanism of attenuation unknown and a risk for reversion to virulence. There is therefore a drive to understand how to rationally attenuate the IBV genome. We have previously generated a recombinant IBV denoted M41-R that is attenuated *in vivo*. Mutations in amino acids in nonstructural protein (Nsp) 10 (Pro85Leu) and Nsp14 (Val393Leu) were associated with attenuation. These amino acids are conserved among members of the coronavirus family and the changes are associated with a temperature-sensitive replication phenotype at 41°C. We investigated the mechanistic action of the identified amino acid changes and the risk of reversion or acquisition of compensatory mutations by serial passages of M41-R, in primary chicken kidney cells and *ex vivo* tracheal organ cultures at 37°C, 39°C and 41°C. Plaque assays of every fifth passage and sequencing were employed to detect changes in viral replication and genetic stability. Changes in cytopathic effects were observed. We have successfully expressed wild-type and mutant versions of the Nsp10 and Nsp14 proteins. Using these we are assessing the impacts of the mutations on Nsp14 associated N7-methyltransferase activity in RNA capping, translation and thermostability.

B017

Development of a novel pseudotyped-virus screening platform to characterise cell entry factors of high consequence infections.

Elle Campbell[1], Thomas Stiff[1], Edward Wright[1], Christine Reitmayer[2], Leandro Castellano[1]

[1]University of Sussex, Brighton, United Kingdom. [2]Keele University, Keele, United Kingdom

Abstract

Development of scalable and highly adaptable platforms to characterise emerging infections are essential for limiting the impact of future viruses with pandemic potential. An understanding of the virus-host interactions is vital, with determining the viral and host factors involved cell entry key for the development of therapeutics and vaccines. Traditional approaches in this field depend on infection assays using authentic viruses that require high containment facilities. However, these are hindered by high running costs, slow processing times and limited scalability. Using pseudotyped viruses (PV) expressing the chikungunya virus (CHIKV) envelope protein as a proof-of-principle, we developed a novel screening platform to identifies cellular factors involved in viral entry. PVs were engineered to express the herpes simplex virus-1 thymidine kinase, which following addition of ganciclovir, can selectively kill infected cells with >90% efficiency. Utilisation of these “killer” PVs in the context of a CRISPR-Cas9 (GeCKO) library gene-knockout A549 cell population permitted selection of cells refractory to infection. Deep sequencing of the selected cells and their comparison to controls using the MAGeCK algorithm, identified novel factors and cellular gene networks involved in CHIKV viral entry, to a clearer resolution compared to authentic viral screens. Drugs targeting these factors inhibited both CHIKV-PV and live CHIKV infections. These results show that our rapid and robust screening platform can identify new therapeutic targets, thereby fortifying global preparations against epidemics and pandemics and making a significant contribution to the “100 days” mission.

B018

DNA packaging of Gene Transfer Agents

Nicholas Read, Paul Fogg, Jason Wilson

University of York, York, United Kingdom

Abstract

Gene Transfer Agents (GTAs) are small phage-like structures produced by some bacteria and archaea. GTAs package random fragments of host DNA into virus-like capsids, with no preference for their own genes, and transfer the DNA to recipient bacteria. The lack of specificity in packaging is thought to be due to unique properties of the packaging motor complex or terminase. Phage terminases are composed of a large terminase subunit (TerL) that carries out enzymatic activities (ATPase and Nuclease) and a small terminase subunit (TerS) that regulates TerL and recognizes the viral genome. GTA TerS have been found to lack the DNA binding domain present in viral TerS and this is thought to lead to lack of target specificity. Meanwhile, GTA TerL contain a number of conserved amino acids that are predicted to be responsible for GTA specific properties, such as unusually low DNA packaging density.

We will present our findings that the GTA TerL and TerS subunits form a $(TerSL)_2$ tetramer complex in solution, in the absence of DNA, and we propose that this could represent an elusive packaging initiation complex. We will show that the small terminase stimulates large terminase nuclease activity and reduces ATPase activity, and that mutations of the conserved residues in both the TerL ATPase and nuclease domains affect GTA activity via altered DNA packaging efficiency. Our data provide new details about how GTA DNA packaging occurs and insights into the differences and similarities to their phage ancestors.

B019

CIDR: Centre for Infectious Disease Reagents – Expanding the portfolio of research reagents to support epidemic and pandemic preparedness for emerging viruses

Robert Cunliffe, Iliana Georgana, Nassim Alami-Rahmouni, Diane Cook, Giada Mattiuzzo, Philip Hogarth, Yann Le Duff

MHRA, Potters Bar, United Kingdom

Abstract

A key component of effective pandemic preparedness is the cost-effective and timely access to high quality research reagents. This accelerates the development of vaccines, diagnostics and therapeutics against emerging infectious diseases, particularly for low and middle income countries (LMICs), which are frequently the most affected. To meet this need, the Medicine and Healthcare products Regulatory Agency (MHRA) has recently launched a new reagent portfolio, the Centre for Infectious Disease Reagents (CIDR). CIDR builds upon the Centre for AIDS Reagents (CFAR), a repository in operation since 1989 that has supplied more than 8500 vials in the past 10 years. CIDR will increase global preparedness and assure an ongoing legacy resource.

Our group has extensive expertise in producing a variety of reagents. Using our successful operating model and expanding network, we are:

- actively engaging with the scientific community to anticipate reagents need.
- encouraging leading scientists to deposit materials to our repository.
- producing and characterising new reagents *e.g.* recombinant proteins, pseudotyped viruses, cell lines and antibodies.
- commissioning new reagents.

The scope of CIDR is guided by priority pathogen lists from UK Vaccine Network, WHO and CEPI, and focuses on Lassa fever, Nipah, Marburg and Ebola diseases, MERS, Chikungunya and others. Materials are made available to institutions worldwide and prioritized to LMICs which benefit from free of charge access. CIDR protects intellectual property rights through material transfer agreements with third party rights. If you have materials that may be beneficial for the wider research community, please contact us at CFAR@nibsc.org

B020

Insights into placental macrophages and how they fight HCMV infection

Naomi McGovern, Emma Poole

University of Cambridge, Cambridge, United Kingdom

Abstract

The placenta is the first organ made by the conceptus and accompanies embryonic development. In humans, placental formation initiates at 5 days post-conception when the blastocyst embeds into the maternal endometrium[1]. Trophoblast cells develop from the trophectoderm surrounding the blastocyst and are the interface between the mother and fetus throughout pregnancy. The multinucleated syncytiotrophoblast (SCT) form the outer layer of the placenta that is resistant to many pathogens. Hofbauer cells (HBC) are extra-embryonic macrophages generated *de novo* within the placenta, first appearing at 18 days post conception. They are the only immune cells normally found in the human placenta throughout pregnancy.

HCMV is the most common congenital infection. It must first cross the placental barrier to infect the fetus *in utero*. Yet, the interaction of HCMV with HBC is relatively poorly understood. A major barrier to progress in this area, has been difficulties in removing contaminating maternal and fetal monocytes/macrophages from HBC preparations.

Our lab has developed techniques to both isolate and culture HBC. We have shown that HBC are derived from placental erythro-myeloid progenitors and not monocytes. These cells have a unique epigenetic profile in comparison with adult macrophages, resulting in distinct properties including a lack of HLA-DR expression.

Ongoing work in the lab involves understanding how the unique properties of HBC impacts on HCMV replication and release.

B022

Investigating the mechanism of coronavirus regulation by m6A RNA modification

Ikshitaa Dinesh ORCID iD, Hannah M Burgess ORCID iD

University of Surrey, Guildford, United Kingdom

Abstract

Human coronaviruses (CoVs), including pandemic β-CoV SARS-CoV-2 and related common cold-causing HCoV-OC43 (OC43), cause respiratory illnesses of varying severity. Recent studies suggest that N6-methyladenosine (m^6A), the most abundant internal modification on eukaryotic mRNAs, plays a key role in regulating CoV infection. M^6A influences critical aspects of cellular mRNA metabolism such as splicing, stability, export, and translation and is installed by the methyltransferase METTL3. Our research previously demonstrated that inhibition of METTL3 with a small molecule inhibitor reduces SARS-CoV-2 and OC43 replication and gene expression, however, the mechanism for this remains unclear. Toward resolving this, we first investigated whether m^6A could regulate CoV infection through differential recognition by innate immune sensors of double-stranded RNA, RIG-I and MDA5. Using targeted gene knockdowns we found that regulation of OC43 by METTL3 is not dependent on RIG-I or MDA5. We further profiled expression of canonical interferon stimulated genes (ISGs) and cytokines reportedly upregulated during OC43 infection and found METTL3 inhibition does not enhance their expression. Though m^6A has been identified on the genomes of human β-CoVs, impaired viral RNA accumulation observed following METTL3 inhibition could be explained by a direct effect on viral RNA or an indirect effect of dysregulation of host RNAs. Here we show that blocking METTL3 activity impacts OC43 RNA accumulation within 5 hours, supporting a direct effect. Using a novel coronavirus RNA decay assay we also show that viral RNA is not destabilized by METTL3 inhibition and are currently investigating effects on viral RNA transcription.

B023

The PLAAT gene family: pro- and anti-viral factors which contribute to virus host range

James Kelly ORCID iD, Soumendu Chakravarti, Andrew Shaw, Chris Neil, Toby Tuthill

The Pirbright Institute, Pirbright, United Kingdom

Abstract

Phospholipase A and acyltransferase (PLAAT) enzymes are a family of lipid-modifying enzymes which in humans consists of 5 members (PLAAT1-5). Human PLAAT3 is known to be an essential host factor for human picornaviruses, such as enterovirus A71 (EV A71). In contrast, we have previously shown that human PLAAT2 and PLAAT4, exhibit antiviral activity against livestock picornaviruses such as foot-and-mouth disease virus (FMDV) but not against human picornaviruses (EV A71). Notably, PLAAT2 and PLAAT4 are the only interferon stimulated genes (ISGs) within the PLAAT family and their presence in mammalian genomes appears to correlate with resistance to FMD. We therefore hypothesize that PLAAT enzymes play a role in determining species susceptibility to FMDV and potentially other picornaviruses. Here using a combination of ectopic expression and siRNA knockdown we have investigated the role of other PLAAT family members from diverse species play in species tropism of viruses.

B024

Investigating molecular mechanisms behind the virulence change from low to high pathogenicity in H7 avian influenza viruses

Kelly Roper ORCID iD[1], Elizabeth Billington[1], Simon Johnson[1], Amanda Seekings[1], Marek Slomka[1], Ashley Banyard[1,2], Joe James[1,2]

[1]Animal and Plant Health Agency, Weybridge, United Kingdom. [2]The Pirbright Institute, Woking, United Kingdom

Abstract

H7 avian influenza virus (AIV) causes a notifiable disease and is one of two influenza subtypes that can mutate in poultry to a high pathogenicity (HP) phenotype. HP emergence is frequently preceded by circulation of low pathogenicity (LP) H7, containing a single-basic cleavage site (SBCS) in the hemagglutinin (HA) glycoprotein. The increased virulence is characterised by acquisition of a multi-basic cleavage site (MBCS) in the HA gene, which facilitates systemic infection with devastating outcomes in poultry. Rare instances of AIVs with a di-basic cleavage site (DBCS) HA are recorded, and previous *in ovo* investigations by our team showed that H7 AIVs with DBCSs mutate to the MBCS. Therefore, DBCS may represent an intermediate during LP to HP evolution. Utilising reverse genetics, a panel of H7 AIVs with SBCS, DBCS and MBCS have been generated, modelled on H7s from past UK poultry incursions of both LP and HP AIVs. The biological properties of these H7 variants *in vitro* will be reported, investigating the impact of amino acid insertions in the CS upon viral fitness. Viral polymerase activity will also be presented, assessing variation in the replicative capacity of LP vs HP AIVs. Future work will generate reassorted H7 AIVs, to study the mutation frequency in the CS, whereby different polymerase gene permutations may drive the switch from LP to HP. Improved understanding of how HP AIV evolves following LP incursion will ultimately inform poultry disease policies and interventions, which are critical to manage and control AIV outbreaks.

B026

Characterisation of Pro-viral picornavirus host factors

Cecilia Dai, Soumendu Chakravarti, Chris Neil, Stephen Berryman, Toby Tuthill, James Kelly ORCID iD

The Pirbright Institute, Pirbright, United Kingdom

Abstract

The picornavirus family includes a large number of small RNA viruses, many of which are significant pathogens for both humans and livestock. For instance, EV-A71 is a major cause of hand, foot, and mouth disease (HFMD) in children, which can lead to hospitalisations with severe complications including acute flaccid paralysis. In livestock, foot-and-mouth disease virus (FMDV), causes a highly infectious condition that results in blisters on the feet and mouths of cloven-hoofed animals (e.g. pigs and cattle) and reduces their productivity. FMDV outbreaks impose substantial costs on the global pig and cattle industries, valued at £700 billion annually. Picornaviruses are therefore a significant burden to both human and animal health, yet the host factors involved in their replication are still poorly understood.

Using CRISPR/Cas9, we have generated stable gene-edited knockout (KO) cell lines to characterise pro-viral host factors required for picornavirus replication. Various methods were employed to generate these gene-edited KO cell lines, with subsequent validation of gene edits through sequencing. These cell lines have been utilised to elucidate the roles these genes play in picornavirus replication. This will provide crucial data that could lead to new strategies to control picornavirus diseases.

B027

Discovery of E3 ligase regulation in the innate immune response to virus infection

Eilidh Rivers ORCID iD[1], Hanna Sowar[1], Arda Balci[1], Satpal Virdee[2], Adam Fletcher[1]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom. [2]University of Dundee, Dundee, United Kingdom

Abstract

The ubiquitin system regulates all aspects of cell biology via a multi-enzyme cascade. The final enzyme in this pathway – the E3 ubiquitin ligase (E3) – catalyses the attachment of the small protein ubiquitin to a substrate, which can be a protein, lipid, nucleotide, or sugar. Many E3 ligases regulate viral infection modifying viral or host proteins to coordinate innate immune defences. Identifying E3 ligases involved in viral replication is a key aim of our lab.

We employed a chemical biology toolkit to monitor for changes in cellular E3 enzyme activities during viral replication. Enzyme activity screening, or 'Activity Based Protein Profiling' (ABPP) requires the construction of semi-synthetic proteins that serve as activity-based probes (ABPs). These E2~Ub ABPs covalently react with E3s containing active site cysteines, a subclass of E3 enzymes. Biotin conjugation of the ABP allows selective enrichment of active E3 ligases from complex cell lysates. Moreover, introduction of ABPs into virally infected cells allows for the capture of regulated E3s in their native environments. Subsequent analysis be mass spectrometry allows identification of E3s that are regulated, positively or negatively, by viral infection. This study will substantially broaden our understanding of the regulation of E3 ligases and provide novel targets to explore the host response to infection.

B028

Development of a WHO International Standard for anti-Sudan Virus Antibodies

Nassim Alami Rahmouni[1], Yann Le Duff ORCID iD[1], Giada Mattiuzzo[1], Emma Bentley[1], Thomas Rudge[2], Chris Cirimotich[2], Julius Lutwama[3], Wendy Boone[4], Joseph Sgherza[4], Michael Selorm Avumegah[5], William Dowling[5], Neil Gibson[2], Larry Wolfraim[6]

[1]Medicines and Healthcare Products Regulatory Agency, Potters Bar, United Kingdom. [2]Battelle, West Jefferson, USA. [3]Uganda Virus Research Institute, Entebbe, Uganda. [4]Integrum Scientific, Greensboro, USA. [5]Coalition for Epidemic Preparedness Initiative, Oslo, Norway. [6]National Institute of Allergy and Infectious Diseases, Rockville, USA

Abstract

Sudan virus (SUDV) causes Sudan virus disease (SVD), a severe illness with a case fatality rate of up to 71%. Compared to the closely related Ebola virus, there is currently no licensed vaccine against SVD, however multiple candidates have entered clinical trials, and several serological assays have been developed. To increase comparability of results generated by these assays, an International Standard (IS) is required. The IS is the highest order of reference material for biological substances and established by the WHO Expert Committee on Biological Standardization (ECBS). The quantification of sample potency using assays calibrated against the IS allows reporting in a common unitage, facilitating data comparison between laboratories, and defining correlates of protection.

To develop the first WHO IS for anti-SUDV antibodies, sera from 28 survivors of the 2000 or 2012 outbreaks of SVD in Uganda were characterised for binding and neutralising activities, in parallel at the MHRA and Battelle. Binding activity, assessed by anti-SUDV glycoprotein IgG ELISA, varied largely between samples, ranging from undetectable to 553.4 ELU/mL. Neutralising activity, assessed using a VSV-based pseudotyped virus assay and expressed as ND50, was relatively low with 93% of the samples found below 200, and ranging from undetectable to 2500. The candidate IS was prepared by pooling the 14 individual sera with the highest binding and neutralising activities and formulated as lyophilised to increase stability. We have recently launched a multi-centre, collaborative study to evaluate this candidate IS and preliminary results that are available will be presented.

B029

Porcine monoclonal antibodies against H5 and H7 influenza virus strains

Tiphaine Cayol[1], Pramila Rijal[2], Sonia Villanueva-Hernandez[1], Adam McNee[1], Kevan Hanson[1], Sylvia Crossley[1], Danish Munir[1], Marie Di Placido[1], John Hammond[1], Basudev Paudyal[1], Elma Tchilian[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom

Abstract

The current H5N1 panzootic has seen an unprecedented spillover into mammals, demonstrating a pressing need for pandemic preparedness against high pathogenicity avian influenza viruses, particularly H5 and H7 subtypes. Monoclonal antibodies (mAbs) targeting the influenza hemagglutinin (HA) can serves as both prophylactics and guide the development of vaccine with broad protection.

Previously we established the pig as a relevant large-animal model to study immunity to influenza virus infection. Furthermore, we demonstrated that porcine influenza hemagglutinin 1 (HA) specific monoclonal antibodies (mAbs) recognize the same HA epitopes as humans. In the present study we isolated mAbs from lymph nodes of pigs immunized with H7 S-Flu and H5- S-Flu, a single cycle replication deficient influenza virus vaccine. H5 and H7-specifc B cells were sorted by FACS, PCR products for heavy and light chains were cloned and expressed in Expi293 cells. The resulting mAbs were purified and screened for binding to recombinant H5 and H7 proteins, H5 or H7 S-FLU or MDCK cells expressing H5 or H7. Promising candidates were selected for large-scale production.

Out of the 48 isolated mAbs, 14 strongly bind and neutralize H5, while 10 do the same for H7. Further work will map their target epitopes as well as assess their cross-reactivity to other H5 and H7 strains. The epitopes recognized by the porcine mAbs will be compared to those seen by humans to establish the utility of the pig for predicting virus evolution and development of novel therapeutic and vaccine strategies.

B030

Screening European Badgers (*Meles meles*) for a novel coronavirus (*Meles*CoV).

Charlotte Parker ORCID iD, Malcolm Bennett, Rachael Tarlinton ORCID iD

University of Nottingham, Sutton Bonington, United Kingdom

Abstract

Previous work into the presence of SARS-CoV-2 in a 2020-2021 UK wildlife demonstrated no detection of this virus in UK wildlife, but did characterise a novel mustelid coronavirus, a previously uncharacterised stoat Minacovirus. Further to this, in 2022 a highly divergent coronavirus (*Meles*CoV) in Italian badgers (*Meles meles*) has been reported, but it is not clear if this virus is present in UK animals or not.

To determine if *Meles*CoV was present in UK badgers, screening of archived UK lung tissue samples was conducted with samples from 2017 to present. The number of samples to be tested was calculated from the prevalence detected in passive surveillance in Italy. UK samples were PCR screened using degenerate coronavirus primers (Drzewniokova *et al*., 2021) that target the RdRp region of the coronavirus genome, highly conserved amongst coronaviruses. Two hundred and eighty lung and thoracic tissue samples were screened from badgers across the UK.

Despite banding following gel electrophoresis being detected in a number of samples, sanger sequencing demonstrated badger DNA indicating non-specific PCR products from these primers in this species. It can be determined that whilst there may be a novel badger virus in mainland Europe, it is unlikely to be present within the UK population at a >1.19% prevalence rate calculated from the surveillance data on *MelesCoV* published.

This work is fundamental to developing our understanding of the diversity and distribution of existing wildlife coronaviruses that are known to jump species between domestic and wild carnivores.

B031

Improving Whole Genome Sequencing of Infectious Bronchitis Virus from in vivo Samples for Characterising Intra-Host Diversity of Live Attenuated Vaccine viruses

Graham Freimanis[1], Ge Wu[1], Charles D'Harrington[1], Tahmina Ruba[1], Noemi Polo[1], Francesca Young[2], Ke Yuan[2], David Robertson[2], Sarah Keep[1], Erica Bickerton[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom

Abstract

Infectious Bronchitis Virus (IBV), an avian coronavirus, poses a significant threat to the global poultry industry. Live-attenuated vaccines (LAVs) are currently the main defence against IBV infections, but these vaccines, have the potential revert to virulence, driving new outbreaks. Standard whole genome sequencing of IBV direct from *in vivo* samples has proven ineffective using established methods due to low viral loads and laborious balancing of primer pools. To investigate intra-host genome diversity of live attenuated vaccine viruses and pathogenic IBV in vivo as they disseminate through hosts, we developed a novel probe enrichment-based assay to characterise genomes of eight different serotypes of Infectious Bronchitis virus.

Optimised molecular probes reliably generated whole genome sequences from *in vivo* samples and molecular virus clones from four serotypes of IBV to coverage depths of more than 10e4 across the IBV genome compared to other methods. We applied the probe assay to investigate dissemination of IBV viruses in both vaccinated and infected hosts. Quantitative RT-PCR screening of organs identified samples for sequencing and aiding mapping of virus dissemination. Virus diversity was characterised in samples from five chickens at three timepoints, infected with either pathogenic or attenuated IBV viruses. We compared whole genome sequence diversity within each host to map common routes of dissemination. Attenuated viruses were less prominent compared to more pathogenic viruses and were present in fewer tissues. Work is ongoing to fully dissect population diversity of whole genome sequences within birds.

B032

A yeast based reverse genetics system of murine hepatitis virus for the study of nsp5 temperature sensitive mutants.

Maximilian Erdmann ORCID iD[1], Rebekah Penrice-Randal[1], Leandro Xavier Neves[1], I'ah Donovan-Banfield[1], Andrew Davidson[2], Edward Emmott[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

Murine hepatitis virus (MHV) is a beta-coronavirus belonging to the subgenus of the *Embecoviruses*. MHV is a useful model system to study coronavirus replication as it does not infect human cells naturally and replicates robustly to high titres in cell culture. Several temperature-sensitive MHV mutants generated by random mutagenesis have been described in the literature. To generate some of these mutants, an infectious clone based on the MHV A59 NCBI: AY700211 genetic background was established using a yeast-based reverse genetics system relying on transformation associated recombination. We assembled a recombinant wild-type and two temperature-sensitive mutants containing non-synonymous F219L changes in nsp5. The DNA genomes were propagated as yeast artificial chromosomes (YAC)s and isolated; sequence verified and used for the generation of capped *in vitro* RNA transcripts for viral rescue. Recombinants were plaque purified and sequence verified. The efficiency of plating of the three recombinants was determined by calculating the ratio of titres achieved by the rescue supernatants at non-permissive and permissive temperatures. Wild-type MHV was not affected in growth while both temperature sensitive mutants showed 1000 -10000-fold growth defects at the non-permissive temperature. Replication characterisation of the recombinants is currently underway. In summary, here we describe the establishment of a yeast-based reverse genetic system for MHV as well as the generation of wild-type and nsp5:F219L temperature sensitive infectious clones to study RNA replication and protein processing.

B033

Mathematical modelling of herpes simplex virus transmission in 2- and 3-dimensions.

Monica Hill ORCID iD, Gillian Elliott ORCID iD, Andrea Rocco ORCID iD

University of Surrey, Guildford, United Kingdom

Abstract

Herpes simplex virus type 1 (HSV-1) has two major routes of transmission in 2-dimensional (2D) cell culture: extracellular (cell-free) and directly across cellular junctions (cell-to-cell), but the relative contribution of each route in host epidermal tissue is not yet understood. Moreover, because the rate and nature of virus spread is a complex interplay of virus and host cell determinants, individual contributions are difficult to assess experimentally. Here, we will present our newly developed 2D stochastic model of HSV1 transmission which combines experimental data using virus replication kinetics and spread of GFP-tagged HSV1 with mathematical modelling. Using Monte Carlo-based simulations, the model incorporates randomness to predict spread characteristics within a single cell monolayer. Following initial infection, the viral load within each cell increases logistically, with the probability of infecting neighbouring cells depending on the viral concentration. Infected cells release virions into the extracellular space, where their diffusion is modelled as a 2D process, occurring throughout the matrix and allowing further infection of the monolayer. The model also incorporates cell death, considering how cellular mortality influences viral propagation and the progression of infection. This model will be used to assess if virus spread occurs in a random or non-random fashion and how immune responses influence virus spread. Ultimately, we will apply our new experimental work on HSV1 infections of 3D-skin rafts to develop a 3D model of virus transmission, enabling us to recapitulate infection dynamics and gain further insights into how HSV-1 spreads within the human host.

B034

Targeting human cytomegalovirus in human kidneys using normothermic machine perfusion technology with the drug candidate SYN002

Erica Appiah[1,2], Sarah Hosgood[2], Johan Lantto[3], Thomas Kledal[3], Michael Nicholson[2], John Sinclair[4], Emma Poole[1,4]

[1]Department of Pathology, University of Cambridge, Cambridge, United Kingdom. [2]Department of Surgery, University of Cambridge, Cambridge, United Kingdom. [3]Synklino A/S, Copenhagen, Denmark. [4]Department of Medicine, University of Cambridge, Cambridge, United Kingdom

Abstract

Human Cytomegalovirus (HCMV) causes minimal harm in the immune competent. However, transplanting a kidney from an HCMV seropositive donor puts an immunocompromised recipient at risk of severe CMV infection and graft failure. SYN002, a fusion toxin protein has been used in vitro to kill CMV infected cells with high efficacy. We investigated the safety and feasibility of the administration of SYN002 to human kidneys during normothermic machine perfusion (NMP). Five pairs of human kidneys were recruited into the study.

One of each pair was treated with SYN002 during NMP and compared to the paired control kidney. Prior to NMP, kidneys were flushed with Ringer's solution and the effluent from the renal vein collected. Perfusate was also collected after NMP. Cells were isolated then cultured and stained for the HCMV immediate-early antigen expression. Perfusion parameters, perfusate, urine and tissue samples were collected for assessment of function and injury.

There was no significant difference in renal perfusate flow [area under the curve (AUC) control 1196 vs treated 1250 ml/min/100g.h, P=0.791] or intrarenal resistance [AUC control 3.00 vs treated 2.4 mmHg/min/100g.h, P=0.366]. There was also no significant difference in injury markers between the treated and control kidneys (P>0.05). In cells collected from the perfusate, the mean foci of infection did not decrease in the control group but was reduced by 95% in the treated kidneys (P=0.01). Thus, SYN002 treatment for HCMV during NMP appeared safe and reduced the foci of infection in cells isolated from the perfusate.

B035

Targeting intersegmental RNA-RNA interactions as a potential approach to optimise the live attenuated influenza vaccine

Ryan Pearce ORCID iD[1], Sameer Ayaz[1], Shaun Cooper[1], Katherine Scott[1], Ziyi Yang[2], Anob Chakrabarti[2], David Bauer[2], Oliver Dibben[1]

[1]AstraZeneca, Liverpool, United Kingdom. [2]The Francis Crick Institute, London, United Kingdom

Abstract

Live attenuated influenza vaccine (LAIV) is an intranasally administered vaccine that protects against seasonal influenza viruses. During the 2015-2016 influenza season, the A/H1N1pdm09 component of LAIV, showed a reduced vaccine effectiveness (VE). A reduced replicative fitness and an increased shedding of non-infectious virus *in vivo* was linked to inefficient genome packaging. Intersegmental RNA-RNA interactions have been recently mapped and postulated to play a role in this process.

Global RNA interaction networks were mapped for the low fitness A/Bolivia/559/2013 (A/BOL13) and the high fitness A/New Caledonia/20/1999 (A/NC99) strains. A high frequency HA interaction was identified in A/BOL13, which was 9-fold lower in A/NC99. Synonymous mutations to disrupt this HA interaction, resulted in 2.2- and 2.4-fold increase in infectious virus titre in A/BOL13 and A/NC99, respectively, suggesting its deleterious nature.

We therefore hypothesised this deleterious RNA interaction may be present in other A/H1N1pdm09 strains and found this region to be highly conserved across A/H1N1pdm09 strains between 2009-2024. To further evaluate this RNA interaction, synonymous mutations were applied to this HA site across a representative panel of A/H1N1pdm09 strains ranging between 2013-2022. Phenotypic changes were observed through remodelling of the RNA interactome. This corresponded to increased infectious virus formation and any changes to genomic composition were assessed.

Overall, these data indicate that RNA interactions play a functional role in LAIV and targeting these could be used during vaccine development.

B036

Impact of SARS-CoV-2 variants on ER stress, genome integrity and their involvement in inflammation

Giuditta De Lorenzo ORCID iD[1,2], Beatrice Pani[1], Pamela Martinez Orellana[2], Alessandro Marcello[2]

[1]Area Science Park, Trieste, Italy. [2]ICGEB, Trieste, Italy

Abstract

An imbalanced inflammatory response plays a key role in severe Covid-19, where multiple inflammatory pathways lead to hyperinflammation and cytokine storm, resulting in acute respiratory syndrome, tissue damage, multi-organ failure, with long-term effects known as Long COVID. Many aspects of the interaction between the virus and the host cell are still to be understood, such as the role that the Unfolded-protein response (UPR) plays during SARS-CoV-2 infection.

SARS-CoV-2 infection induces virus-induced DNA damage (VIDD) through Orf6/NSP13 mediated degradation of CHK1 and Nucleocapsid-mediated impaired 53BP1 recruitment at the site of damage, leading to activation of proinflammatory pathways and virus-induced senescence (VIS) with autocrine/paracrine activity.

Recent variants of concern (VoCs), including currently circulating Omicron sub-lineages, have shown recurrent substitutions in Orf6, N and NSP13, and enhanced expression of Orf6 and N including the production of a novel subgenomic RNA encoding a truncated form of N. The effect of these changes on virus-induced DD and senescence in the context of inflammation and both acute and chronic pathogenesis remain poorly characterized. We plan to determine the impact of SARS-CoV-2 VoCs on VIDD and VIS, particularly focusing on their implication in paracrine neuroinflammation.

B037

Development of Assays to Measure the Humoral Response Against Mpox

Stephen Laidlaw ORCID iD[1,2], Tom Tipton[1,2], Yasmin Jiwa[1], Maria Lima[1], Miles Carroll[1,2]

[1]University of Oxford, Oxford, United Kingdom. [2]Pandemic Sciences Institute, Oxford, United Kingdom

Abstract

Historically mpox has only been seen in Africa, with the occasional sporadic exportation to other countries. All this changed in 2022 when Clade IIb mpox began rapidly spreading worldwide, particularly in the MSM community. This was followed by a novel clade, Ib, which has recently emerged in Central and East Africa. These latest outbreaks are characterised by increased human to human transmission, primarily following close or intimate contact.

As an increased number of people become exposed to mpox it is important to understand any risk factors and the overall population susceptibility to infection. We have addressed this shortfall in two ways. Firstly, we have established an Orthopoxvirus ELISA to study serum antibody concentrations and secondly, we have developed a neutralisation assay to detect Orthopoxvirus neutralising antibodies.

Using sera samples from a blood donor cohort we have established a background cut-off, which has allowed us to determine antibody levels in both convalescent individuals and patients enrolled in mpox vaccination studies.

Additionally, we have been able to show that the Orthopoxvirus ELISA clearly delineates those older individuals who received the smallpox vaccination in their childhood, before the eradication of smallpox was declared in 1980 by the WHO.

B038

Integrating molecular assays with viral genomics to identify fitness factors driving lineage turnover of foot-and-mouth disease virus.

Natasha Edwards[1,2], Selma Schmidt[1], Noemi Polo[1], Jemma Wadsworth[1], Soumendu Chakravarti[1], Valérie Mioulet[1], Emma Taylor[2], Donald P. King[1], Dan L. Horton[3,2], Lidia Lasecka-Dykes[1], Andrew E. Shaw[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]The University of Surrey, Guildford, United Kingdom. [3]The Animal and Plant Health Agency, Weybridge, United Kingdom

Abstract

Foot-and-mouth disease virus (FMDV), the causative agent of foot-and-mouth disease (FMD), is a highly transmissible RNA virus, affecting cloven-hoofed animals of the order Artiodactyla. FMD infection results in high levels of morbidity, with severe clinical signs including lesions, pyrexia and lameness. FMDV circulates globally and results in severe economic losses to affected countries. FMDV has evolved into seven antigenically distinct serotypes, the most widespread and prevalent of which is serotype O. Each FMD serotype circulates in the form of multiple genetic lineages, with new lineages frequently emerging. Whilst the mechanistic basis for lineage success remains unknown, occasionally new lineages emerge and come to dominate on a larger scale, resulting in FMD panzootics. The primary aim of this study is to identify potential genome-encoded viral fitness factors contributing to lineage turnover and the emergence of panzootic strains. Here, we characterised multiple examples of four serotype O lineages, representing recent panzootic and non-panzootic strains at the genetic and phenotypic level. Differences were observed *in vitro* in immortalised cells, but these appeared not to correlate with a panzootic phenotype. In contrast, growth curves undertaken in primary bovine kidney epithelial (PBKE) cells showed differences in viral growth kinetics, potentially suggesting a more robust innate immune system is a driver of FMDV phenotype. To begin dissecting the molecular mechanisms underpinning lineage turnover, we combined competition assays with nanopore technology sequencing. These results demonstrate that approaches undertaken *in vitro* can be used to interrogate questions arising from observations at an epidemiological scale.

B039

Alphavirus Capsid Protein Interactome

Elizaveta Bratslavskaia[1], Andres Merits[2], Margus Varjak[1]

[1]Institute of Technology, University of Tartu, Tartu, Estonia. [2]Institute of Bioengineering, University of Tartu, Tartu, Estonia

Abstract

Alphaviruses (family *Togaviridae*), spread by mosquitoes in (sub)tropical regions, present a significant challenge and highlight the need for developing novel antiviral drugs. Understanding the interactions of viral proteins with proteins in human host and insect vector cells can reveal potential cellular pro- and antiviral factors, which may serve as focal points for designing effective treatments.

In the study, we explored the interactome of the Chikungunya virus (CHIKV) capsid protein (CP), which is a key player in virus replication, virion assembly, and virus-cell interactions. A quantitative, label-free proteomics analysis was conducted to investigate CHIKV CP interactors in two types of cells- human host and mosquito vector cells. This approach enabled us to detect common proteins and pathways essential for viral infection in two evolutionarily very distant species, highlighting their biological significance.

Several host and vector factors were identified, among them were many homologous human and mosquito proteins. The impact of selected interactors on CHIKV pathogenesis was assessed in mosquito and human cells by conducting gene silencing, followed by infection with a nanoluciferase-expressing virus. By screening, multiple proteins were found to be proviral in both cell types, demonstrating the CHIKV CP need for functional conservation across species and providing insights into the molecular mechanisms underlying CHIKV pathogenesis.

Future research will involve the expression of capsid proteins from various alphaviruses to investigate their interactome in human and mosquito cells, aiming to identify similarities across different members of this viral genus.

B040

Assessing distinctiveness profiling for predicting SARS-CoV-2 variant success

A S M Rubayet Ul Alam, Keiran Lamb, Joseph Hughes, David L. Robertson

MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

Abstract

SARS-CoV-2 variants are emerging constantly as this new human coronavirus continues to evolve. A key challenge is to predict which variants could be dominant in the future. An important focus of current research is thus assessing the potential impact of those novel variants *in silico*. The measure of genetic distance from previous variants that have circulated in a geographical region, i.e., 'distinctiveness' of a new variant, can potentially capture the past immunological exposure to the human population. Previous research (PMID: 35899067) supported 'distinctiveness values' use in genomic surveillance. Here, we test the use of distinctiveness with a view to predicting the emergence of new variants in a country. We first show that despite the large amount of SARS-CoV-2 sequencing data the heterogeneous nature of sampling in the pandemic requires more consistent sequencing for surveillance purposes. We then find that there is no strong correlation between distinctiveness values and subsequent prevalence of novel variants at 14-days intervals over a 56-days window. Highly prevalent variants circulated in all the countries showing variations in distinctiveness values. In contrast, variants that are only prevalent in specific countries would have significantly higher distinctiveness value in other countries. Our results demonstrate that the distinctiveness value of SARS-CoV-2 variants alone cannot define a novel variant's success or failure. Further research is required to disentangle virological aspects of success from epidemiological factors.

B041

Pharmaceutical inhibition of stress granule formation during Infectious bronchitis virus (IBV) infection dampens the innate immune response and promotes viral replication

Sam Kelly[1,2], Helena Maier[1], Nicolas Locker[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]The University of Surrey, Guildford, United Kingdom

Abstract

The cellular innate immune response needs to be tightly regulated upon viral sensing to limit viral replication while maintaining cell homeostasis. The phase separation of biomolecules in condensates, such as stress granules (SGs) has recently been implicated in regulating these processes. While SGs are formed as part of the integrated stress response, emerging data shows that many viruses actively inhibit the formation of SGs or even hijack SG proteins. While this suggests an anti-viral role for SGs, the importance of this role and the underlying mechanisms are under debate.

Our aim is to determine the importance of SG formation during Infectious bronchitis virus (IBV) infection. Previous data show that while IBV broadly inhibits SG formation, 20% of infected cells still display SGs. By completely abolishing SG formation we aim to address the role of SGs in context of a SG-disruptive infection model. To achieve this, we used the small molecule inhibitor G3Ib that targets the major SG scaffolding protein G3BP and has previously been shown to prevent the formation of sodium arsenite induced stress granules. Frist using immunofluorescence we showed that G3Ib completely abolish SG formation during IBV infection in Vero cells. Further we found that treatment with G3Ib suppresses the innate immune response which is associated with an increase in viral replication. Similar results were obtained during infection of chicken DF1 cells, resembling the natural host of IBV. These results show that G3Ib can inhibit viral SGs and highlights an anti-viral role of SGs in IBV infection.

B042

Mannose-Binding Lectins as Effective Antivirals Against SARS-CoV-2 and Emerging Variants

Victoria Grosche ORCID iD[1,2], Igor Santos ORCID iD[3], Claudener Teixeira ORCID iD[4], Mark Harris ORCID iD[1], Ana Carolina Jardim ORCID iD[5]

[1]University of Leeds, Leeds, United Kingdom. [2]São Paulo State University, São José do Rio Preto, Brazil. [3]Pirbright institute, Pirbright, United Kingdom. [4]Federal University of Cariri, Cariri, Brazil. [5]Federal University of Uberlandia, Uberlandia, Brazil

Abstract

The COVID-19 pandemic emphasized the urgent need for effective antiviral therapies targeting SARS-CoV-2 and its emerging variants. In this regard the Brazilian flora is rich in bioactive compounds which offer antiviral potential. An attractive target for antiviral intervention is the Spike glycoprotein which mediates the entry of SARS-CoV-2 into host cells, mainly through the ACE2 receptor. Due to the high glycosylation of these proteins, carbohydrate-binding agents such as lectins may represent potential candidates to abrogate virus infection. Here we evaluated lectins extracted from 4 Brazilian plant species: Canavalia maritima (ConM), Canavalia ensiformis (ConA), Machaerium acutifolium (MaL), and Parkia platycephala (PPL), which exhibit mannose binding affinity. Lectins ConM, ConA, MaL, and PPL inhibited replication of a SARS-CoV-2 derivative expressing an mNeongreen reporter with selective indices of 155.5, 81.6, 118.8, and 24.7 respectively. All stages of viral infection were inhibited. Preincubation of virus with ConM, ConA, and MaL reduced infectivity by 96% while PPL reduced infectivity by 71.1%. Intriguingly viral RNA synthesis was also reduced by lectin treatment (80% reduction for all except PPL (49.5%). Activity was observed against Delta and Omicron variants. The presence of exogenous D-(+)-mannose eliminated the anti-SARS-CoV-2 activity of the lectins, restoring viral titres. These findings suggest that mannose-binding lectins, especially ConM, ConA, and MaL are potent agents against SARS-CoV-2, mainly by blocking viral entry. However, they may also affect later stages in infection, highlighting their potential as a basis for new antiviral drugs targeting SARS-CoV-2 and its variants.

B043

Generation of African Swine Fever Virus Pseudotypes

George Hooper ORCID iD[1], Chris Netherton[2], Edward Wright[1]

[1]Viral Pseudotype Unit, School of Life Sciences, University of Sussex, Brighton, United Kingdom.
[2]The Pirbright Institute, Ash Road, Pirbright, United Kingdom

Abstract

African swine fever (ASF) is a severe haemorrhagic contagious disease of Eurasian wild boar and domestic swine, which exhibits an exceptionally high mortality rate, usually 100% in acute infection. This is in part because the virus replicates to high titres before the host can mount an effective immune response. Moreover, there are no approved treatments or vaccines, although, several are in development. Therefore, the virus has been able to spread rapidly, causing the deaths of millions of pigs and massive economic losses; crippling the pig industry and threatening biodiversity.

The causative agent is a large, complex DNA virus that encodes for over 150 proteins and can be transmitted via multiple mechanisms (fomite, direct contact, soft-bodied ticks (sylvatic cycle)). Our understanding of ASFV is still limited in some areas, such as, which viral and cellular factors are involved in cell entry. We have sought to address this by attempting to generate pseudotyped viruses (PV) to study this stage of replication.

Based on published data and *in silico* analysis, a set of ASFV proteins potentially involved in cell entry were identified. These proteins were cloned, and their expression and incorporation onto the surface of PV was assessed by immunofluorescent microscopy. The ability of PV bearing combinations of these proteins to infect susceptible cells was then assessed by infection studies.

ASFV pseudotypes would greatly improve our ability to identify host and viral factors involved in cell entry, crucial for researchers developing treatments and vaccines to combat this deadly disease.

B044

Improving Influenza Serology Accessibility through Dried Blood Spot Sampling or sera collected on a filter paper and a Lyophilized H1-H18 Pseudotyped Virus Kits

Tobias Mapulanga[1,2], Maria Giovanna Marotta[3], Kelly A S da Costa[1,2], Simon D Scott[1,2], Nigel Temperton[1,2]

[1]University of Kent, Kent, United Kingdom. [2]University of Greenwich, London, United Kingdom. [3]University of Siena, Siena, Italy

Abstract

Traditional hemagglutination inhibition (HI) assay, commonly used to evaluate influenza vaccines, has significant limitations. These include low sensitivity, reproducibility issues stemming from red blood cell variability in sialic acid specificities, and an inability to detect HA stem antibodies, rendering the HI assay inadequate for assessing universal influenza vaccines. To overcome these challenges, we have developed a lyophilized H1-18 panel of influenza A pseudotyped viruses with titres ranging from 2.1x 10^7 to 4.81 x10^{10} RLU/ml. This panel enables safe, sensitive and more comprehensive evaluation of influenza vaccines, immunotherapy, and sero-surveillance, and is especially suited for low- and middle-income countries where stringent cold chain requirements and shipping costs limit access. The use of lyophilized pseudotyped viruses helps reduce these barriers, and dried blood spots or sera collected on filter paper—demonstrating a recovery rate of approximately 50%—offer the additional benefit of simplifying sample collection and transportation logistics. In parallel with a select panel of SARS-CoV-2 VOCs within a dual-neutralisation assay format we propose a multi-site collaborative study to validate the efficacy of this novel approach, with the goal of making influenza and coronavirus serology more accessible, cost-effective, and comprehensive worldwide.

B045

Identification of non-canonical open reading frames through large scale screening of rotavirus genome segments

Hou Wei Chook, Blanka Tesla, Lu Lu, Paul Digard, Eleanor Gaunt

The Roslin Institute, University of Edinburgh, Midlothian, United Kingdom

Abstract

Due to the high error rates of RNA-directed RNA polymerases, most RNA virus genomes are constrained in size. To expand their coding capacities, RNA viruses sometimes encode polycistronic genes accessible through non-canonical gene expression mechanisms, such as alternative translation initiation and out-of-frame open reading frames (ORFs). Rotaviruses are known to encode additional protein species through such mechanisms. To identify ORFs that could encode additional protein species, we developed computational pipelines that use two definitions of ORFs. These are: (1) sequences that do not contain in-frame stop codons; and (2) sequences flanked by in-frame start and stop codons, where overlapping ORFs are permitted. Both pipelines identify the canonical reading frame of each inputted sequence automatically, eliminating the need for prior knowledge of the canonical reading frame. We analysed large sequence datasets of rotavirus genome segments to identify conserved non-canonical ORFs. We identified a conserved upstream start codon (uAUG) in the rotavirus NSP3 segment that if utilised, could produce an elongated version of NSP3. We generated mutants of the SA11 rotavirus strain targeting the NSP3 uAUG and canonical start codon. Removal of the NSP3 uAUG appears to increase the rate of cell death during infection independently of growth kinetics. Removal of the canonical start codon however, generates an attenuated mutant. Finally, we identified a conserved uAUG in the NSP1 segment absent in SA11, and are currently generating a mutant where the uAUG is introduced.

B046

Genomic characterisation of influenza A viruses in the natural environment: A One Health approach

Aaron G. Canton Bastarrachea[1], Andrew Lee[1], Damien Tully[2], Ceri Fielding[3], Deirdre Gilpin[1], Kenneth Lemon[4], John McGrath[1], Connor Bamford[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]London School of Hygiene & Tropical Medicine, London, United Kingdom. [3]Cardiff University, Cardiff, United Kingdom. [4]Agri-Food and Biosciences Institute, Belfast, United Kingdom

Abstract

The persistent pandemic threat of avian influenza A viruses (IAV) has been recently evidenced by the emergence and spread of novel, highly pathogenic viruses – particularly of the of H5 subtype – among wild and domestic mammals across the Americas, with several reported human infections. Wild birds and their aquatic environments play a crucial role in the maintenance and spread of IAV, including highly pathogenic subtypes. Understanding IAV circulation and evolution in its avian and environmental reservoirs is thus necessary to prevent and control outbreaks before they spillover to other animals and humans.

Recent evidence from wastewater studies, including our own research, highlight the potential for environmental surveillance to detect and characterise IAV genomes from human and animal sources. However, the applicability and integration of such approaches to more complex matrices, such as those from natural environments, has not been fully explored. This study aims to adapt wastewater genomic surveillance methods to improve the recovery of IAV genomes across diverse environmental specimens, enabling a better understanding of IAV risk over time and space.

Preliminary results suggest strategies to strengthen the sensitivity and robustness of IAV detection and sequencing in wetland sediments by enhancing the stability of viral RNA, mitigating PCR inhibitors, and exploiting improved genomics approaches. We will present the potential application of this to monitoring of wild bird habitats in Ireland. Altogether, our work supports the potential utility of environmental studies to help prevent and control pathogenic threats with a One Health approach.

B047

Investigating the role of conserved RNA motifs within the 3’UTR of coronaviruses

Rupa Tripathy[1,2], Ciaran Gilbride[1], Ziyi Yang[1], David LV Bauer[1]

[1]The Francis Crick Institute, London, United Kingdom. [2]University of Oxford, Oxford, United Kingdom

Abstract

RNA virus genomes possess cis-acting sequences and structural elements in their untranslated regions (UTRs) that are critical for processes in the viral life cycle that influence transmission, such as RNA synthesis. The 3'UTRs of coronaviruses contain conserved structures such as a bulged-stem loop and adjacent pseudoknot, but also a downstream hypervariable region (HVR) with a more complex secondary structure and reduced sequence conservation. Interestingly, an octanucleotide motif (GGAAGAGC) is conserved in almost all coronavirus 3'UTRs and resides within this otherwise hypervariable region. The strict conservation suggests an important role, but no function for the motif has been elucidated to date.

Using the seasonal human coronavirus OC43 (HCoV-OC43) and a novel reverse genetics toolkit, we produced viruses with tags and modifications to the HVR: we deleted the entire HVR, deleted and modified the octanucleotide motif and related sequences, and modified secondary structures within the HVR to understand if these motifs are essential. We then evaluated how these modifications impacted viral replication in-vitro using various techniques, including RT-qPCR, viral genome sequencing by tiling amplicons, and plaque assays.

Overall, our work enhances our understanding of the roles of conserved RNA sequences and structural elements in coronavirus replication. These findings will inform future in-vivo studies on how the coronavirus 3'UTR influences pathogenesis.

B048

The role of non-structural protein 2 in porcine reproductive and respiratory syndrome virus induced immunosuppression.

Bailey Hairsine[1,2], Shahriar Behboudi[2], Simon Graham[1], Rory Fortes De Brito[1]

[1]The Pirbright Institute, Pirbright, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

Porcine reproductive and respiratory syndrome (PRRS) is an infectious disease characterised by reproductive failure, respiratory distress, and secondary bacterial and viral infections. The current understanding is that the causative PRRS viruses (PRRSV) mediate immunosuppression of the host, however, the underlying mechanisms involved are unclear. The upregulation of cyclooxygenase 2 (COX-2) may be influential in this process as the resulting downstream activation of cyclic adenosine monophosphate negatively regulates the T-cell receptor mediated activation of effector T-cells. The PRRSV non-structural protein 2 (NSP2) is a highly variable, multifunctional protein that has previously been implicated in the upregulation of COX-2. Therefore, we are investigating the role of NSP2 on the COX-2/prostaglandin E2 pathway and how mutations within the NSP2 gene may influence the virulence of PRRSV strains through modulation of the porcine immune response. Using bioinformatic analysis, we evaluated the link between conserved motifs within the NSP2 encoding open reading frame and differential pathogenicity of PRRSV strains. Using this data, we are comparing the effects of different PRRSV strains and their NSP2 on the upregulation of COX-2 and anti-inflammatory cytokines, such as interleukin-10, tumour growth factor-β1, and integrin $\alpha_v\beta_8$. This research should enhance our understanding of the role of PRRSV NSP2 on the induction of immunosuppression during infection. This is critical in advancing the poor safety and immunogenicity of current live attenuated vaccines.

B049

Harnessing Nature's Pharmaceutical Toolbox: Exploring Plant-derived Compounds and Synthetic Xanthones to Combat Coronaviruses

Gemma Cooper[1], Bethanie Dean[1,2], Tim Snape[3], Maitreyi Shivkumar ORCID iD[1]

[1]Leicester School of Pharmacy, De Montfort University, Leicester, United Kingdom. [2]Department of Chemistry, University of Warwick, Warwick, United Kingdom. [3]Faculty of Health and Life Sciences, De Montfort Univerisity, Leicester, United Kingdom

Abstract

In the last 20 years three pandemic coronaviruses have emerged, with many more coronaviruses circulating in animal reservoirs, viral emergence is likely. There is a need for broad-spectrum antivirals which can be deployed in response to emerging viruses.

Plant extracts have been used around the world for medicinal purposes and are a rich source of novel antiviral compounds. We evaluated a panel of compounds and synthetic xanthones based on *Swerita* bioactive compounds for antiviral activity against human coronaviruses HCoV-OC43 and HCov-229E. Infected cell lines were treated with the compounds, and impact on infectivity assessed by quantifying viral titres.

We identified six natural product compounds displaying antiviral activity at non-cytotoxic concentrations, with potential broad-spectrum activity demonstrated across two model coronaviruses .

Although synthetic xanthones significantly reduced viral infectivity, their potency was lower than that of the natural xanthone mangiferin, which showed a ≥3 log10 reduction in infectivity. The additional structural complexity of mangiferin and other natural xanthones may contribute to their enhanced antiviral efficacy.

Mechanism of action studies suggests that the hit compounds inhibit the virus at early stages in the viral lifecycle. However, the compounds do not show inhibition of spike pseudo-typed lentivirus infection, showing that spike-mediated entry is unlikely to be the inhibitory mechanism. Our preliminary data indicate potential inhibition of the viral main protease, and further studies are underway to confirm this target.

This work provides insights into the potential for identifying and modifying natural compounds, offering a promising pathway towards new pan-coronavirus antiviral development.

B050

Investigating potential for anti-NA cross reactivity via vaccination and natural infection

Kelly da Costa ORCID iD[1], Maria Giovanna Marotta[2], Joanne Del Rosario[3], Serena Marchi[2], George Carnell[4], Claudia Trombetta[2,5,6], Jonathan Heeney[4,3], Emanuele Montomoli[2,5,6], Nigel Temperton[1]

[1]University of Kent, Kent, United Kingdom. [2]University of Siena, Siena, Italy. [3]DIOSynVax, Cambridge, United Kingdom. [4]University of Cambridge, Cambridge, United Kingdom. [5]VisMederi Research srl, Siena, Italy. [6]VisMederi srl,, Siena, Italy

Abstract

Influenza A viruses (IAV) express both Hemagglutinin (HA) and neuraminidase (NA) on the surface of virus particles, both are immunogenic but the traditional focus of vaccine responses has been to HA. Despite the fact NA remains present in vaccines, although at unknown quantities, therefore contribution to the protection offered by anti-NA responses following vaccination or prior exposure is unclear.

To understand the impact of anti-NA antibodies, drugs and future vaccine candidates, sensitive and robust assays are required. We have previously developed a pseudotyped virus (PV) library of N1 - N9 PVs and a PV ELLA assay (pELLA).

To explore how NA inhibition may contribute to pandemic preparedness we tested anti-NA drugs in pELLA against N1-N9. We vaccinated mice with different strains of NA to test the ability of polyclonal antibodies to inhibit across strains and subtypes from N1-N9. We also assessed sera responses in individuals who have been exposed to seasonal influenza strains which have circulated over the last 100 years including 1918 H1N1 and H2N2 IAV.

Evidence suggests that previous NA exposure is beneficial. Moreover, humans are exposed to NA as part of current seasonal vaccine, the viral life cycle during natural infection and exposure over their lifetimes. Licenced IAV drugs such as Oseltamivir target NA to lessen disease severity by preventing viral dissemination in the host. We show here how NA inhibition could play an important part in protective immunity as part of a universal vaccine and provide further evidence that NA responses should be considered moving forward.

B051

Cloning and Analysis of an Epstein-Barr virus Strain from an LCL Established with virus from Kenyan Breast Milk.

Anrong Li[1], Luke Granger[1], Wiyada Wongwiwat[1], Amr Bayoumy[2], Rob White[1]

[1]Imperial College London, London, United Kingdom. [2]University of Coventry, Coventry, United Kingdom

Abstract

The majority of in vitro studies of EBV biology have relied on a small number of EBV strains chat carry mutations through cancer evolution or prolonged cell culture. It is therefore important to ensure our understandings of EBV biology are valid for EBV strains representative of normal circulating EBV. There is therefore a need to establish infection models for circulating strains from different geographic regions.

We have developed a new protocol for cloning EBV genomes. This uses cas9-induced homologous recombination to integrate a loxP-flanked BAC into the EBV genome between BVRF1 and BVLF1, recoding overlapping parts of the ORFs to avoid repetitive DNA flanking the BAC. Five days post transfection, we extract total genomic DNA from the cells, and then enrich the EBV DNA using a biotinylated EBNA1 DNA-binding domain. Enriched DNA is transformed into bacteria to recover the BAC-cloned EBV genome.

This strategy can reproducibly clone Jijoye EBV from its parental cell line. To enable cloning of EBV from LCLs, we co-transformed the BAC capture vector with cas9-RNP, cloning an intact EBV genome from an LCL (BM209) established by infecting B cells with breast milk from a Kenyan individual (Daud et al 2014). Both Jijoye and BM209 BACs were used to generate 293 virus producer cell lines. These both generated low levels of encapsidated EBV genomes, that were increased approximately 200-fold when a cre-expression vector (to excise the BAC element) was included alongside the virus-induction, suggesting that BAC-cloned EBVs may otherwise exceed the efficient packaging limit of the virus.

B052

Investigating molecular interactions driving tick-borne virus persistence in *Ixodes ricinus* tick cells

Rees Sorby[1], Lesley Bell-Sakyi ORCID iD[2], Marine J. Petit ORCID iD[1]

[1]University of Surrey, Guildford, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Tick-borne encephalitis virus (TBEV), a causative agent of severe neuropathology in central and western Europe, poses a significant public health threat. Its persistence within tick vectors, particularly *Ixodes ricinus*, elevates the risk of transmission in endemic areas. This project investigates molecular interactions that facilitate TBEV's long-term survival in tick cells, combining experimental approaches with molecular modelling.

To understand how tick-borne flaviviruses interact with tick proteins to induce long-term infection, virus-tick interactions in *Ixodes* cells were studied using V5-tagged viruses. Due to the challenges of working with flaviviruses, circular polymerase extension reaction (CPER) was used to produce V5-tagged tick-borne flaviviruses, including TBEV and Langat virus (LGTV). An innovative approach was taken to optimise tag placement using AlphaFold to minimise functional disruption, overcoming limitations associated with traditional N- and C-terminal tagging.

Next, engineered V5-tagged flaviviruses were characterised in *Ixodes ricinus* and mammalian cell lines. Growth kinetics were assessed by measuring viral titres via plaque assay, and RNA replication by RT-qPCR, establishing standard methods for persistent infection studies in *Ixodes* cells. To confirm V5-tag stability, immunostaining of persistently infected tick cells was performed. Altogether, this tick-virus system and the tools developed represent major advancements in studying tick-virus protein interactions. Coupled with affinity purification-mass spectrometry (AP-MS), this work will reveal conserved and species-specific mechanisms influencing viral stability and maintenance in tick vectors. The research expands understanding of flavivirus-vector interactions, providing foundational knowledge for strategies to control the spread of tick-borne flaviviruses and reduce health risks in endemic regions.

B053

The role of non-structural protein 1 (NS1) in Influenza A virus host adaptation and host shutoff

Miki Uchima[1], Ruth Harvey[2], Jon Wilson[3], Anob Chakrabarti[4], Steve Gamblin[3], Hannah Mischo[5]

[1]King's College London and Francis Crick Institute, London, United Kingdom. [2]Worldwide Influenza Centre; Francis Crick Institute, London, United Kingdom. [3]Francis Crick Institute, London, United Kingdom. [4]University College London, London, United Kingdom. [5]King's College London, London, United Kingdom

Abstract

The non-structural protein 1 (NS1) of influenza A virus (IAV) is a key factor in antagonizing the host antiviral response by targeting cellular proteins within cytoplasm and nucleus. Nuclear NS1 sequesters CPSF30, a crucial factor for polyadenylation site recognition, resulting in host transcription termination defects (TTDs). This ultimately causes transcriptional host shutoff.

NS1-CPSF30 binding is highly conserved in human-adapted IAVs which suggests an evolutionary advantage in the human host. This has been supported by studies of the swine flu pandemic strain A/California/07/2009 (CA07). While CA07 NS1 does not interact with CPSF30, NS1-CPSF30 binding is positively selected for during subsequent adaptation to the human host. However, reports of NS1-independent mechanism of IAV-mediated host shutoff question how NS1-CPSF30 interaction contributes to IAV virulence and host shutoff.

Here we show that CA07 infection induces TTDs in human and swine lung epithelial cells, despite the inability of CA07 NS1 to block gene expression. Furthermore, we present an optimised cell fractionation protocol to characterise nuclear and cytoplasmic interactors of human/swine NS1 of IAV-infected human/porcine lung epithelial cells.

Lastly, we show that polymerase intact nascent transcript (POINT) sequencing can be performed on IAV-infected cells. This nascent transcriptomics approach will be used to study global consequences of different NS1-CPSF30 interactions on viral host shutoff.
Combined proteomics and transcriptomics datasets will contribute to our evolutionary and mechanistic understanding of NS1 and its role in host adaptation. This may improve risk assessment of newly emerging influenza strains and aid prediction accuracy of virus evolution in different hosts.

Session : Microbial Warfare: conflicts between species, strains, and mobile genetic elements

B055

It's Lyse To Meet You: Introducing the *Pseudomonas* 43A Tailocin to *Escherichia coli* O157:H7

Audrey Sweten ORCID iD, David Baltrus ORCID iD

University of Arizona, Tucson, USA

Abstract

Escherichia coli O157:H7 is a major cause of foodborne illness in the USA and the UK, often occurring due to consumption of agricultural products colonized with *E. coli* O157:H7. Tailocins are bactericidal protein complexes produced by bacteria that kill closely related bacteria strains with specificity and precision. It is thought that tailocins target bacteria via binding to the lipopolysaccharide, disrupting the outer membrane potential, and resulting in cell lysis of the target bacterium. Recently, we screened *Pseudomonas* strains for tailocin production and sensitivity against various human-relevant pathogens. In this screen, we identified a bacterial strain, *Pseudomonas* 43A, which had tailocin-like activity against *E. coli* O157:H7. Therefore, we hypothesized the use of tailocin killing activity as the mechanism behind *Pseudomonas* 43A strain killing activity of *E. coli* O157:H7. To characterize the genetic basis of the novel tailocin-activity produced by *Pseudomonas* 43A, we deleted the tailocin sheath, a gene that controls tailocin production, via homologous recombination and tested for the absence of *E. coli* O157:H7 killing via an overlay assay. We found that the *Pseudomonas* 43A tailocin was responsible for *E. coli* O157:H7 killing activity and targeting of a multidrug-resistant strain of *Pseudomonas aeruginosa*. We hypothesize that this tailocin works collaboratively with a bacteriocin to kill other closely related strains. We also explored the physical properties of the killing activity, including induction by Mitomycin C, thermotolerance/cryotolerance, and protein destruction via Proteinase K. Future studies aim to explore the prophylactic use of this novel tailocin to combat bacterial infections in agriculture.

B056

Genome Mining Reveals Novel Antimicrobials and Skin Microbiome Fitness Factors of *Staphylococcus epidermidis*

Keir Nicholas-Haizelden[1], Holly Richardson[1], Roy Goodacre[1], Barry Murphy[2], Malcolm Horsburgh[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Unilever, Port Sunlight, Birkenhead, United Kingdom

Abstract

Staphylococcus epidermidis is the most ubiquitous and abundant *Staphylococcus* of the human skin microbiota. Through the production of a variety of secondary metabolites, *S. epidermidis* contributes to skin homeostasis using various mechanisms, such as humectant liberation, local immune system priming and pathogenic colonisation resistance. Specialisation and adaptation to variations in skin environments by *S. epidermidis* is highlighted through high strain level diversity between bodily sites and individuals. Given the large and open pangenome of *S. epidermidis* containing many undefined genes, factors underpinning *S. epidermidis* fitness remain largely uncharacterised.

In vitro growth inhibition assays coupled with genome-wide biosynthetic gene cluster mining tools has revealed a wealth of secondary metabolites potentially contributing to species fitness. In this study, experimental coevolution has revealed specific antimicrobial peptides exerting considerable intraspecific selective pressure on susceptible strains revealing candidate genetic loci of *S. epidermidis* associated with novel antimicrobial entry and resistance mechanisms. Further competition between antimicrobial non-producers and non-antagonistic quorum sensing systems of *S. epidermidis* revealed genetic loci associated with potential cellular fitness factors that remain uncharacterised. Improved understanding of how *S. epidermidis* competes within the skin environment is necessary for informing the development of novel skin relevant treatment strategies and personal care products.

B057

From Petri Dishes to Soil: Streptomyces on the Hunt for Antimicrobials and Beyond.

Alejandra Diaz de Aguinaga ORCID iD, Lorena Fernández Martínez ORCID iD

University of Glasgow, Glasgow, United Kingdom

Abstract

Since the culmination of the Golden Age of antimicrobial discovery, scientist worldwide have been on a race against emerging Antimicrobial Resistance (AMR) and the discovery of new antimicrobial classes. Due to their success, the widely recognised antimicrobial-producing genus *Streptomyces* has been central in this search. But is the classical approach for antimicrobial discovery enough to win this race? This research proposes a "back-to-soil" strategy, returning *Streptomyces* to their natural niche to study their physiological responses to environmental and community pressures, aiming to understand the social dynamics of *Streptomyces* in soil and its association with natural product production. This research will focus on studying the interactions between *Streptomyces spp.* and the well-characterised three-strain system THOR (The Hitchhikers of the Rhizosphere), in chemically defined media, soil and soil extract. This will be followed by the evaluation of changes in microbial abundance and phenotype, and the quantification of physicochemical parameter changes. We will then conduct metabolomic analyses to investigate the effect of community interactions on secondary metabolite production, and transcriptomic analyses to evaluate the shifts in gene expression.

Studying the interactions between *Streptomyces spp*. and other environmental bacteria will contribute to fill in the gaps for the prediction of silent BCGs inducers. Furthermore, understanding *Streptomyces* in this broader context beyond its capacity to produce natural products can contribute to open new areas of research into developing solutions to problems in other fields such as agriculture and bioremediation.

B058

Characterising the role and evolution of temperate bacteriophages in chronic respiratory infections of Pseudomonas aeruginosa in Cystic Fibrosis (CF) patients using their first isolate.

Mariklairi Kiourkou[1], Mohammad Adnan Tariq[1], Roger Levesque[2], Malcolm Brodlie[3], John Perry[4], Audrey Perry[4], Emma Marrs[4], Andrew Nelson[1], Darren Smith[1]

[1]Northumbria University, Newcastle Upon Tyne, United Kingdom. [2]University of Laval, Quebec, Canada. [3]Royal Victoria Infirmary, Newcastle Upon Tyne, United Kingdom. [4]Freeman Hospital, Newcastle Upon Tyne, United Kingdom

Abstract

Cystic fibrosis (CF) is an autosomal recessive genetic disorder marked by impaired mucociliary clearance and persistent lung inflammation, that leads to chronic bacterial infections. Pseudomonas aeruginosa (Pa) infection is associated with increased morbidity and mortality in CF patients due to its ability to adapt and evolve in the lung microenvironment. Temperate phages, integrate into the P. aeruginosa genome and play a functional role in bacterial adaptation and evolution within the CF lung environment. This study aims to characterize these temperate phages as potential markers of bacterial evolution by investigating how they influence P. aeruginosa physiology and examining their carriage at the onset of chronic infection or longitudinally. Understanding phage-driven genetic changes could provide insights into the pathophysiology of CF lung infections, particularly during the transition from early to chronic infection stages. Using a set of clinical isolates (n=328) provided by the Freeman Hospital, Newcastle. These were sequenced using a 384-indexing strategy, using the PacBio Revio platform. Analysis confirms the use of long-read sequencing at high plexity to both assemble Pa genomes and mine for prophage carriage. This research seeks to highlight the differences in bacteriophage genome complexity carriage associated with late-stage lung infections in CF, contributing to a deeper understanding of bacterial adaptation and chronic infection in this patient population.

Overall, the project aims to establish that temperate phage provides selective advantages in Pa and this arms-race evolution contributes to the chronic infection in the CF lung.

B059

Understanding the role of Multifaceted Defense Mechanisms of *Acinetobacter* spp.

Saadlee Shehreen ORCID iD

Tesside University, Middlesbrough, United Kingdom

Abstract

Acinetobacter spp. poses significant challenges in clinical settings due to its extensive antibiotic resistance mechanisms such as β-lactamase production, which deactivates β-lactam antibiotics, efflux pumps that expel antibiotics from the bacterial cell, alterations in antibiotic target sites, and biofilm formation that protects bacterial communities. Additionally, Tn7-like transposons contribute to the formation of resistance islands, enhancing antibiotic resistance. The comprehensive analysis of anti-phage defense mechanisms in *Acinetobacter* spp. provides critical insights into optimizing phage therapy. This study aims to elucidate the anti-phage defense mechanisms of *Acinetobacter* spp. to develop more effective phage therapy strategies. By investigating the prevalence of various defense families and horizontal gene transfer (HGT) traits, we seek to optimize phage therapy and mitigate the emergence of bacterial resistance variants. The synergistic interactions among different defense families, which may confer both immediate resistance and long-term immunity by minimizing phage escape potential. Understanding these interactions is crucial for developing phage therapy strategies that can effectively counteract bacterial resistance. Here, we analyzed over 100 *Acinetobacter* spp. isolates to examine the prevalence of diverse defense mechanisms, including CRISPR-Cas, and other newly identified systems. Additionally, we assessed HGT traits such as heavy metal resistance, antibiotic resistance, and integrative conjugative elements (ICE). By understanding bacterial evolution in response to phage attacks and the role of synergistic interactions among defense families, we can develop more robust and effective treatments to combat this challenging pathogen.

B060

Gene transfer agents in *Brucella anthropi*

David Sherlock ORCID iD, Paul Fogg

University of York, York, United Kingdom

Abstract

Gene transfer agents (GTAs) are defective phages that can package and transfer random pieces of the producing cell's genome, but are unable to transfer the genes required for their own production. Previously we showed that a specific GTA activation factor (gafA) is essential for GTA production in *Rhodobacter capsulatus* (RcGTA) and this allowed us to understand how multiple host sensor/regulatory systems are integrated to control production of RcGTAs.

Our further aim was to investigate GTA activity on species beyond the Rhodobacter model species species in other potential GTA producing species that contain gafA homologues, including those from the *Brucella* and *Ochrobactrum*. The *Brucella* gafA genes have also been implicated as virulence/fitness factors of unknown function in high-throughput studies. However, the several mechanistic details remain unclear. Here we present our current progress in the work to investigate these mechanisms.

Interuption of the genes that potentially control GTA synthesis have produced interesting phenotypes which have altered their growth and sensitivity to antibiotics. These results were quantified by qPCR, RNAseq and GTA transfer assays.

Our results advance our understanding of this fascinating mode of horizontal gene transfer, not only in the model species but also in other potential GTA producing species that contain gafA homologues.

B061

Exploring the effect of bacteriophage infection on *Ralstonia pseudosolanacearum* transcriptome

Daniel Narino-Rojas ORCID iD[1], Ville Friman ORCID iD[2,1], Paul Fogg ORCID iD[1], Andrea Harper ORCID iD[1]

[1]University of York, York, United Kingdom. [2]University of Helsinki, Helsinki, Finland

Abstract

Ralstonia pseudosolanacearum (Rs) is a devastating plant pathogen that causes bacterial wilt disease, leading to huge economic losses worldwide. Moreover, there are no effective plant protection measures against this pathogen, making it imperative to develop novel control approaches. Bacteriophages (phages) are promising biocontrol agents for Rs: they are highly target specific, propagate rapidly in soil, and efficiently reduce bacterial densities. Bacteria possess vast arsenals of anti-viral defence systems that could compromise phage bactericidal efficiency. However, knowledge of the genetic mechanisms and dynamic gene expression of defence systems and virulence genes during phage-bacteria interactions is limited for Rs. We have conducted liquid infection assays on Rs using taxonomically and morphologically distinct phages from our collection. We found bacterial density dynamics vary considerably when Rs is challenged by different phages, suggesting that different host responses are activated, e.g. defence systems, depending on the specific phage they are exposed to. Based on our preliminary phage infection results, we performed an RNA-seq analysis of the transcriptome of Rs under infection for 13 hours by three different phages. We will present data on which bacterial genes are differentially expressed in response to each phage, and analysis on which genes are associated with generalized or specific responses to phage infection stress. This experiment will shed light on key genes playing major roles on bacterial fitness during phage infection and the impact of taxonomy and morphology of different phages on the bacterium transcriptomic response, increasing the knowledge on Rs-phage interactions in efforts to improve phage biocontrol efficacy.

B062

Investigating how expression levels affect the protection provided by *Pseudomonas aeruginosa* anti-phage defense systems.

Meg Llewellyn ORCID iD, Aleksei Agapov, Edze Westra, Stineke Van Houte

University of Exeter, Penryn, United Kingdom

Abstract

Bacteriophages are viruses that can infect, kill, and alter bacteria. Existing in a 5-10-fold excess to bacteria, phages create a strong selection pressure for bacteria and subsequently have driven the evolution of an arsenal of anti-phage defence systems (DSs). Researchers at the Westra lab previously modified *Pseudomonas aeruginosa* strain PAO1 by removing all known anti-phage DSs and introduced individual DSs on plasmids to assess their function against a panel of diverse phages. This revealed an unexpected, extensive lack of protection, leading to the question of whether the DSs are functional, and what conditions may affect the protection levels they provide. To test this, we have replaced the DSs native promoters with inducible promoters to allow us to see how overexpression affects the system's functionality. Additionally, we plan on testing the effects of phage load and temperature on protection levels. This work broadens the understanding of the functionality and protection levels of *Pseudomonas aeruginosa* defence systems.

B063

The antimicrobial activity of copper sulphate as an intestinal habitat filter affecting *Salmonella* and the pig gut microbiota interactions

Rafal Kolenda[1], Marwa M. Hassan[2], Ainhoa Arrieta-Gisasola[3], Mark Stares[4], Hilary Brown[4], Gwenaelle Le Gall[5], Abioseh Kamara[5], Alfredo Chavez-Arroyo[6], Rebecca Ansorge[1], Trevor Lawley[4], Andreas J. Baumler[6], Roberto La Ragione[2], Falk Hildebrand[1], Robert A. Kingsley[1]

[1]QIB, Norwich, United Kingdom. [2]University of Surrey, Guildford, United Kingdom. [3]University of the Basque Country, Vitoria-Gasteiz, Spain. [4]Sanger Institute, Hinxton, United Kingdom. [5]University of East Anglia, Norwich, United Kingdom. [6]UC Davis, Davis, USA

Abstract

Colonisation of pigs by *S.* Typhimurium is a major factor for transmission to humans. During colonisation of pigs, *Salmonella* encounters a hostile environment, including the resident microbiota and antimicrobials such as copper. Copper sulphate is used as a feed additive in the pig industry since EU-wide ban on the use of antibiotics as growth promoters. The most recent pandemic *S.* Typhimurium clone acquired genomic island SGI-4 encoding copper resistance genes. Copper may act as intestinal habitat filter affecting the microbiota and providing colonisation advantage for copper-resistant *Salmonella*. Hence, we tested the hypothesis that high copper in feed affects development of pig gut microbiota and competition between *Salmonella* and other microbes in 4–6-week-old piglets. Differences between high and low copper diet were observed for the 14 species including *Bifidobacterium*, *Escherichia*, and *Lactobacillus* considered as probiotics. As *Enterobacterales* have been previously shown to compete with *Salmonella* for gut colonisation, 180 isolates were cultured comprising over 170 *E. coli*. Long-read sequencing of selected *E. coli* revealed the presence of copper resistance clusters integrated mainly into one chromosomal locus but on a mobile genetic element distinct from copper-encoding SGI-4 in *S.* Typhimurium. *In vitro* assays revealed that presence of copper resistance is pivotal for competition between *Salmonella* and *E. coli* to occur in high copper supplementation, but there are additional factors that provide *Salmonella* advantage over *E. coli* in copper-formed niche. Copper supplementation substantially modifies the gut microbial community of piglets that have the potential to alter competition between pathogenic and commensal bacteria.

B064

Interactions between *P. aeruginosa* and *S. aureus* in CF are lineage-specific

Sadhbh Dodd ORCID iD[1], Dr Siobhán O'Brien[1], Dr Jo Fothergill[2]

[1]Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College Dublin, Dublin, Ireland. [2]Institute of Infection, Veterinary and Ecological Sciences, Faculty of Health & Life Sciences, University of Liverpool, Liverpool, United Kingdom

Abstract

Cystic fibrosis (CF) is a common recessively inherited disease characterised by consistent chronic lung infections. Two key pathogens colonizing the CF lung are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. While *S. aureus* is the dominant pathogen infecting patients during childhood, *P. aeruginosa* often appears by early adulthood, displacing or occasionally co-existing with *S. aureus.*

The ability *of P. aeruginosa* to outcompete *S. aureus* and dominate the CF lung is a worrying milestone in the life of a patient with CF, as *P. aeruginosa* infections are associated with worsening disease symptoms and mortality. Hence, understanding the factors that allow *P. aeruginosa* to outcompete or coexist with *P. aeruginosa* is key for managing disease progression.

Here, we perform in-depth analysis of a collection of 40 *P. aeruginosa* isolates from a single CF patient sample. By preforming reciprocal supernatant and plaque assays, we show that the interactions between *S. aureus* and *P. aeruginosa* are lineage-specific – so different within-patient lineages are affected by *S. aureus* in distinct and opposing ways. Our work suggests that the use of a wider range of clinical isolates is necessary to understand the role of interspecies interactions for predicting the evolution *of P. aeruginosa* in CF.

B065

Elucidating the phageome in the developing gut of preterm infants

Enrique Gonzalez Tortuero ORCID iD[1], Wen Chyin Yew[1], Jasmine Jessop[1], Rui Nunes Dos Santos[1], Gregory Young[2], Lauren C Beck[2], Andrea C Masi[2], Christopher J Stewart[2], Nicholas D Embleton[2,3], Andrew Nelson[1], Janet E Berrington[2,3], Darren Smith[1]

[1]Northumbria University, Newcastle upon Tyne, United Kingdom. [2]Newcastle University, Newcastle upon Tyne, United Kingdom. [3]Newcastle upon Tyne Hospitals National Health Service Foundation Trust, Newcastle upon Tyne, United Kingdom

Abstract

The intestinal microbiota is, among other factors, a relevant player in developing complex, critical diseases in adults and neonates. In preterm infants, those less than 28 weeks gestational age, microbial colonisation and subsequent disequilibria within the gut correlate to the risk of developing life-threatening complex diseases such as necrotizing enterocolitis (NEC) and late-onset sepsis (LOS). However, little is known about the composition and abundance of gut bacteriophages with the establishing microbial communities of these neonates and how each child's gut phageome compares to the microbiota present in NEC and LOS. In this study, we aim to compare the viral diversity and composition of 1,874 preterm stool samples taken from 178 infants, taken during 8 weeks, with deep metagenomic sequencing that has been resolved for bacterial taxonomy. Bacteria were assembled and mined for phage species. Of the 3,283 retrieved putative bacteriophages, 3,055 were not currently described, having those phages less than 70% ANI with currently known and classified phages, despite the current methodological limitations of prophage and shorter predicted phage mining tools. Such a catalogue will be used to test the impact of temperate bacteriophages in NEC and LOS. Additionally, to understand the role of these temperate phages in pathogenesis, we will assess the presence of antimicrobial resistance (AMR) genes and virulence factors in this novel phage catalogue.

B067

SXT/R391 Integrative and Conjugative Elements (ICEs) and Their Relationship with *Proteus mirabilis* Hosts

Douglas Lyra H. Fonseca ORCID iD, Rodrigo S. Galhardo ORCID iD

University of São Paulo, São Paulo, Brazil

Abstract

The association of mobile genetic elements (MGEs) with the rise of multidrug-resistant bacteria has become increasingly concerning; however, the complexity of the relationship between these elements and their hosts requires further investigation. SXT/R391 Integrative and Conjugative Elements (ICEs) are MGEs that integrate into the chromosome and can be transferred horizontally, spreading antimicrobial resistance genes, mainly among members of the *Vibrionaceae* and *Morganellaceae* families. Our study aimed to evaluate aspects of the relationship between ICE*Pmi*Jpn1, one of the most widely disseminated SXT/R391 ICEs, and its natural host, *Proteus mirabilis*. For this investigation, we used isogenic strains (containing or not the ICE*Pmi*Jpn1), which allowed us to assess the influence of this element on physiological and pathogenic aspects of *P. mirabilis*, and variations in ICE transfer through conjugation, competition and qPCR assays. We found that ICE*Pmi*Jpn1 did not impact the fitness, self-recognition, pathogenicity and persistence ability of this bacterium, increased biofilm formation in one strain and slightly reduced swarming motility. Additionally, ICE transfer varied significantly across *P. mirabilis* donor strains, independent of competitive ability, but associated with levels of excised circular ICE and the expression of genes involved in conjugation regulation. The results indicate that ICE*Pmi*Jpn1 has minimal effects on the physiology or pathogenicity of *P. mirabilis*, reflecting a good relationship between this element and its host. Furthermore, our findings suggest that strain-specific factors, rather than species-specific, modulate ICE transfer between bacteria. This work was supported by CAPES – Funding Code 001 and FAPESP grants no. 2020/00535-5, 2021/15170-5, and 2022/03986-3.

B068

Determining the regulation of the newly discovered anti-phage defence system MADS.

Jasmine Thomas-Campbell ORCID iD[1,2], Aleksei Agapov ORCID iD[2], Anna Olina[2], Stineke Van Houte ORCID iD[2], Tiffany Taylor ORCID iD[1], Edze Westra ORCID iD[2]

[1]University of Bath, Bath, United Kingdom. [2]University of Exeter, Exeter, United Kingdom

Abstract

Bacterial defence systems play a role in the spread of antimicrobial resistance and offer protection against phage infection. Recent work has demonstrated that a newly discovered eight gene defence system MADS cooperates with CRISPR-Cas to protect *Pseudomonas aeruginosa* cells from phage infection. Such a complex system must be tightly regulated by a yet unknown mechanism. Bioinformatic analysis currently suggests that the *mad1* gene from the MADS operon is a transcriptional repressor involved in its regulation.

To test the role of mad1 in MADS regulation we are using a panel of knockout strains derived from a clinical isolate of *Pseudomonas aeruginosa* (SMC4386). Deletion of single genes from MADS operon allows us to assess their role in regulation. We developed a reporter system based on *mCherry* gene under the control of putative MADS promoter to monitor its activity in the knockout background. We examined the effect of *mad1* on expression levels of MADS immune systems across different environmental and growth conditions (high density, low density, lytic, lysogenic, clonal, mixed).

In this work we aim to confirm the role of *mad1* as a negative regulator of a novel defence system MADS, investigate how environmental conditions and phage infection triggers the defence, and if MADS and CRIPSPR-Cas system are co-regulated. This work will reveal how bacteria manage their immune systems to balance fitness costs with the benefit of protection. In the age of antimicrobial resistance this work can shed light on predicting and manipulating phage resistance levels in bacterial pathogens.

B069

Bacteriophage therapy for the treatment of Diabetic Foot Ulcers - exploring the use of phage cocktails as alternative antimicrobial treatment of infected DFUs.

Elspeth Smith ORCID iD[1], Graham Stafford[1], Dave Partridge[1,2], Tom Darton[1]

[1]University of Sheffield, Sheffield, United Kingdom. [2]Sheffield Teaching Hospitals, Sheffield, United Kingdom

Abstract

Antimicrobial resistance (AMR) presents a significant global public health challenge, rendering current therapies ineffective and posing life-threatening risks to those afflicted by infections from multidrug-resistant organisms, such as diabetic foot ulcers (DFUs) —with approximately 20% culminating in amputations. Phage therapy, harnessing viruses abundant in the biosphere to combat bacteria, emerges as a promising antimicrobial alternative.

In this study we aimed to: i) Isolate and characterise a collection of phages that target common DFU pathogens; ii) Develop a phage cocktail that can inhibit the growth of DFU pathogens; iii) Explore the use of this cocktail in synergy with antibiotics.

Novel phages were isolated from the environment (wastewater) and characterised based on morphology (TEM) host range and genome sequencing. Their host ranges were determined and quantified by Efficiency of plating (EOPs) assays. At present, we have isolated over 60 novel phages, belonging to different taxonomic groups and infecting a range of clinical DFU strains. These include phage against clinical strains of the ESKAPE pathogens; *Enterococcus* spp., *Staphylococcus* spp., *E. coli, Klebsiella* spp. and *Enterobacter* spp. Host range data combined was used as a basis of cocktail development - targeting a mixture of DFU clinical isolates (often isolated from the same patient). Liquid killing assays with target pathogens were used to assess the efficiency of phage cocktails and observe emergence of phage resistance. Combinational treatment of phage cocktail and antibiotics was employed to investigate any synergistic effects that could enhance treatments of polymicrobial AMR infections and improve NHS diabetic foot ulcer care.

B070

UNDERSTANDING THE MOLECULAR MECHANISMS OF SPONTANEOUS PHAGE RESISTANCE AND PHAGE-DRIVEN CAPSULE VARIATION IN *KLEBSIELLA* SPP.

Deus Kamya ORCID iD[1,2,3], Hannah Pye[1,3], Keith Turner[1,3], Claire Elek[1,3], Emma Holden[1,3], Muhamad Yasir[1,3], Mark Webber[1,2,3], Evelien Adriaenssens[1,3]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom. [3]Centre for Microbial Interactions, Norwich, United Kingdom

Abstract

Klebsiella is a genus of gram-negative opportunistic pathogens which inhabit the nasopharyngeal and intestinal tracts of healthy individuals. They are frequently isolated in hospital-acquired infections and account for 19% of total blood stream infections in the UK. Of immediate concern, however, is the ever-increasing number of antibiotic-resistant strains, due in part to the capsule—a hypervariable polysaccharide-based layer encompassing the cell wall. Among other things, the capsule is also critical for phage infection—enabling and sometimes inhibiting phage adsorption. Although phage therapy has been explored for treatment of *Klebsiella*-associated infections, we still do not fully understand the breadth of phage-host interactions and their impact on virulence characteristics like capsule variation.

Using phage plaque assays, we observed spontaneous emergence of phage-resistant colonies with altered morphologies, suggestive of capsular differences, and preliminary sequence analyses show mutations in the capsule locus genes as a likely cause of phage resistance. We therefore hypothesise that virulent phage infection is a strong driver of capsule variation in *Klebsiella.* To understand the underlying molecular mechanisms, we are utilising Transposon Directed Insertion-site Sequencing (TraDIS) to simultaneously identify host genes involved in both phage infection and capsule synthesis. We have identified candidate strains of *K. michiganensis* and *K. pneumoniae* infected by at least ten taxonomically distinct phages. A transposon mutant library has been constructed in the *K. michiganensis* strain to identify host genes essential for phage infection.

Our findings will contribute to the understanding of phage-host evolutionary dynamics and provide valuable insights for optimizing phage therapy against *Klebsiella* infections.

B071

Pangenomics of *Staphylococcus aureus*: insights into intra-species gene flow and the evolution of lineages

Jamie Gorzynski ORCID iD[1], Josh Harling-Lee ORCID iD[1], Wendy Figueroa-Chavez[2], Gonzalo Yebra ORCID iD[1], Tom Freeman ORCID iD[3], José Penadés ORCID iD[2], Ross Fitzgerald ORCID iD[1]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]Imperial College London, London, United Kingdom. [3]Janssen Immunology, Philadelphia, USA

Abstract

Bacterial population and pangenome structures depend on the frequency and extent of Horizontal Gene Transfer (HGT). However, our understanding of the levels of intra-species HGT and its impact on population structure is incomplete. The multi-host pathogen *Staphylococcus aureus*, responsible for an array of human and animal diseases, has a population differentiated into distinct lineages (or Clonal Complexes [CCs]), each containing unique restriction-modification barriers to DNA uptake. However, the dynamics of gene flow within and between CCs across the species diversity have not been previously examined.

Here, we employed a combined bioinformatic analysis of 4,000 *S. aureus* genomes with experimental testing of HGT in vitro to examine variation in pangenome structure, gene sharing and barriers to HGT across the *S. aureus* species. We identified extensive variation between lineages in patterns of gene distribution and exchange. In particular, the pangenome openness of CCs was strikingly varied, ranging from closed to very open. For CCs with closed pangenomes, there was reduced gene sharing with most other CCs in the analysis marked by the presence of unique R-M systems. Notably, the bovine-restricted CC151 had the most closed pangenome and reduced genome size marked by genome degradation. Contrastingly, the promiscuous multi-host CC398 lineage had the most open pangenome and exhibited high levels of gene sharing with most other CCs.

Taken together, our analysis provides new insights into the pangenome dynamics of *S. aureus*, revealing remarkable lineage-dependent variation in HGT dynamics consistent with the central influence of defence systems on intra-species gene flow and population structure.

B072

Exploring Prophage Contributions to Bacterial Competition and Adaptation in *Pseudomonas aeruginosa*

Xinyan Yu[1,2], Anna Olina[1], Stineke van Houte[1], Edze Westra[1]

[1]University of Exeter, Penryn, United Kingdom. [2]Nanjing Medical University, Nanjing, China

Abstract

Bacteriophages, viruses that infect bacteria, are the most abundant organisms on Earth. Among them, virulent phages typically cause bacterial lysis and release progeny phages. In contrast, temperate phages can integrate into the bacterial genome to form prophages, which replicate along with the host bacteria rather than immediately lysing the cells. This integration has been shown to affect the host's phenotypes, such as adaptability and competitiveness, but the underlying mechanisms remain poorly understood.

In this study, we used the opportunistic pathogen *Pseudomonas aeruginosa* as a model to investigate the role of prophages in bacterial competition and adaptation. We isolated temperate phages from a collection of *P. aeruginosa* clinical isolates and used each phage to generate lysogens (prophage-bearing strains) in a *P. aeruginosa* laboratory strain. We then assessed the phenotypes of these lysogens, focusing on their competitive fitness against other strains as well as their adaptability including biofilm formation and motility. Additionally, we performed genome sequencing on selected lysogens to identify the sequences of integrated temperate phages and their integration sites within the bacterial genome. Currently, we are integrating these sequence data with phenotypic changes of lysogens to explore the underlying mechanisms, which could enhance our understanding of the role of prophages in bacterial competition, adaptation, and evolution.

B073

See it, Say it, Sort it: Unravelling Indole's Influence on Interspecies Communication and Salmonella Survival Strategies"

John L Clark-Corrigall ORCID iD[1], Bethany Gollan[1], Heather Allison[2], Anjam Khan[1]

[1]Newcastle University, Newcastle upon Tyne, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

Salmonella spp. are recognised as one of the top four global causes of diarrhoeal disease, with many infections originating from both omnivorous and plant-based diets. Notably, some strains of *Salmonella* have now evolved to be resistant to multiple antibiotics, presenting a growing challenge to public health.

Motility is essential to *Salmonella's* physiology, enabling it to navigate the gut environment through chemotaxis, which in turn assists in locating a niche within the host. Bacteria frequently encounter environmental changes and must be able to detect and adapt to stimuli, a function they achieve, in part, through two-component systems.

To cause infection, *Salmonella* must overcome colonisation resistance, a natural defence mechanism whereby the host's intestinal microbiota prevent invading pathogens from establishing themselves and causing disease. Numerous members of the microbiota, including probiotic strains, actively defend their niche by producing antagonistic compounds, one of which is the small aromatic molecule, indole.

Indole influences core bacterial traits, including physiology, virulence, and biofilm formation. While indole's effects have been documented in various pathogenic bacteria with some understanding of its detection mechanisms, its impact on *Salmonella* physiology and virulence is still poorly characterised

In this study, we investigate the effects of both exogenous and probiotic cell-culture-derived indole concentrations on the motility of two key *Salmonella* strains, SL1344 and the bloodstream invasive D23580, offering new insights into how these bacteria detect and respond to indole. Our findings have important implications for understanding *Salmonella's* adaptive strategies within the environment of the gastrointestinal tract.

B074

The Oral Mobilome in Caries and Periodontitis

Jack Lynch[1], David Bradshaw[2], Ricarda Hawkins[2], Rob Howlin[2], Sue Pavitt[1], Thuy Do[1]

[1]University of Leeds, Leeds, United Kingdom. [2]Haleon Consumer Healthcare, Weybridge, United Kingdom

Abstract

The human oral microbiome has been extensively studied for its composition and functions. However, the oral mobilome, encompassing mobile genetic elements (MGEs) that facilitate horizontal gene transfers, remains underexplored. MGEs play a pivotal role in microbial evolution, antibiotic resistance, and virulence. To understand its impact on health and disease, we investigated adolescents with caries and oral health, alongside periodontitis-suffering and orally healthy adults using publicly available whole-genome sequencing data.

MEGAHIT and PLASMe were used to construct contigs and analyse plasmid content, while WAAFLE detected horizontal gene transfer (HGT) events. R packages DESEQ2 and phyloseq assessed differential abundance and diversity changes. Identified plasmids were analysed for protein content using the NCBI nucleotide database.

Analysis revealed significant differences in the mobilome between health and disease. Plasmid analysis highlighted 6 enriched and 2 depleted plasmids (padj<0.05 & Log2FC > +/- 0.5) in the caries cohort. Enriched plasmids originate from species not commonly found in the mouth, including those from soil (*Rothia terrae*), equine/sheep (*Streptococcus equinus*), chicken (*Campylobacter jejuni*), and gut (*Limosilactobacillus gastricus)*, highlighting the importance of mobilome monitoring from a One Health perspective. HGT analysis revealed key donor-recipient pairs, with skewed networks in disease involving caries/periodontitis-associated genera. Interestingly, GCYS-20, a host gene enriched in periodontitis samples associated with gastric-cancer development, gives evidence of host-microbe interactions.

In conclusion, this study provides valuable insights into the oral mobilome, describing plasmids associated with caries and periodontitis compared to health. Current research aims to delve deeper into plasmid functions and explore MGE insertion characteristics.

B075

How do interactions between plasmids and transposons affect resistance gene spread?

Victoria Orr ORCID iD[1], Calvin Dytham[2], Ellie Harrison[3], James Hall[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]University of York, York, United Kingdom. [3]University of Sheffield, Sheffield, United Kingdom

Abstract

Horizontal gene transfer (HGT) is a core driver of rapid bacterial evolution, and plasmids are key to this process, spreading adaptive traits such as resistance genes. It is increasingly clear that plasmid-borne resistance genes are usually 'nested' on transposons, enabling mobilisation and carriage by plasmids. This web of interactions (plasmid-transposon-chromosome) is predicted to have important implications for the spread of resistance genes. Using a laboratory microcosm system and computer modelling, we investigated how different plasmid 'vehicles' affected the spread of a chromosomal, transposon-borne resistance gene and how the transposability of a trait can affect single species populations. We found that resistance gene mobilisation varied across a panel of plasmids largely independent of conjugation rate, suggesting that other plasmid features, such as gene content or presence of other transposons, may influence chromosomal gene mobilisation. To test the contribution of intra-genome gene mobility to the persistence and spread of traits, we constructed plasmids carrying mobile (i.e. on a transposon) and non-mobile (i.e. integrated in the plasmid backbone) resistance genes, and tested the effects on MGE persistence under varying environmental selection regimes. To more broadly explore how plasmid features (conjugation rate, maintenance cost) combined with transposon features (transposition rate) affect resistance gene spread, we developed an agent-based model to disentangle the complexities of these interactions and predict how trait spread is affected in a variety of environmental scenarios. Understanding the potential outcomes and consequences of interactions between MGEs has important application in predicting the evolution of traits like antimicrobial resistance in microbial communities.

B076

The speed of host range evolution depends on microbial community composition and environmental context

Dibyendu Dutta ORCID iD[1], Gretel Waugh ORCID iD[1], Emma Tollafield[1], Wolfram Wolfram Möbius ORCID iD[1,2]

[1]Living Systems Institute, Faculty of Health and Life Sciences, University of Exeter, Exeter, United Kingdom. [2]Physics and Astronomy, Faculty of Environment, Science and Economy, University of Exeter, Exeter, United Kingdom

Abstract

Bacteriophages shape microbial communities by controlling bacterial population sizes and facilitating horizontal gene transfer. They do this with high specificity determined by their host range, which is not static but can change through evolution. The dependence of bacteriophage host range evolution on biotic and abiotic context is poorly understood. We studied host range expansion of bacteriophage T7 from wild-type E. coli to strains that are originally resistant to T7 due to knockout of genes of the LPS biosynthesis pathway: E. coli ΔwaaC and ΔwaaR. We performed short-term evolution experiments of T7 and compared the rate of bacteriophage evolution across a range of host community compositions at low and high population density both in liquid and on solid media. Combining fluorescence microscopy with high-throughput miniaturised PFU assays, we contextualised phenotypic outcome (i.e., ratio of efficiency of plating on both hosts) with qualitative observations of infection within the host community. As expected, host range expansion was more pronounced in all communities containing the new host. Unexpectedly, environmental context and type of new host changed evolution qualitatively: Phage evolved rapidly to infect E. coli ΔwaaC in a lawn on solid media, but not in liquid. The same did not hold true for new host E. coli ΔwaaR. Our results highlight the role of spatial organisation of microbes in bacteriophage evolution. They call for a systematic exploration into how organisation of the host community with respect to biofilm characteristics including metabolic state and spatial structure shape phage evolution in nature and in clinical applications.

B077

Look who's talking: Mapping the network of crosstalk between Arbitrium phages

Rebecca Woodhams[1], Robyn Manley[2], Edze Westra[1]

[1]University of Exeter, Penryn, United Kingdom. [2]University of Exeter, Exeter, United Kingdom

Abstract

Bacteriophages are the most abundant biological entities on the planet. Temperate phages choose between two lifecycles upon infection; lytic, where they lyse their host to release new virions, or lysogenic where they integrate into the bacterial genome. SPβ-like phages utilise a communication system similar to quorum sensing to coordinate this decision upon infection of their Bacillus host. The concentration of so-called Arbitrium signal molecules indicates density of infected hosts, allowing the infecting phage to make an appropriate lifecycle decision.

Closely related phages produce different signal molecules. Canonically, it was believed that phages could only sense their own signal. However, recent data from our lab has shown that the infection dynamics of phi3T (an SPβ phage) are affected by non-cognate signals, which we are terming "crosstalk". Therefore, arbitrium has potential for being a mechanism of cooperation or competition between Bacillus phages.

Here, we investigate the specificity or promiscuity of these systems. Utilising a collection of both natural and chimeric phi3T phages, we are establishing a communication network between these phages. Preliminary results indicates that there may be potential of crosstalk for Goe11. Evolution experiments are also underway to determine whether phage can rapidly evolve towards or away from crosstalk, and the molecular steps involved in this.

Together, these experiments will reveal which arbitrium receptors are capable of crosstalk, allowing us to form conclusions as to when crosstalk may be beneficial or maladaptive to a phage. Jointly, this work will show the relevance of crosstalk to competition between closely related bacteriophages.

B078

Exploring phage-mediated adaptation of *Ruminococcus gnavus* to the human gut

Xena Dyball[1,2], Ryan Cook[1], James Docherty[1,2], Emmanuelle Crost[1], Nathalie Juge[1], Evelien Adriaenssens[1]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]University of East Anglia, Norwich, United Kingdom

Abstract

Ruminococcus gnavus is a prevalent member of the human gut microbiota and over-represented in individuals suffering from gastrointestinal disorders, such as inflammatory bowel disease. *R. gnavus* strains show strain-specific differences in their mechanisms of adaptation to the gut. However, little is known about the bacteriophages (phages) associated with this species and their role in influencing *R. gnavus* strain-level diversity and phenotypes. Here, we developed a custom bioinformatic pipeline to mine the presence of prophages across 79 *R. gnavus* strains. The results showed that prophages are prevalent among *R. gnavus* genomes, with 75% of strains encoding at least one prophage. Taxonomic analyses indicated that the prophages grouped into 78 unique species clusters, largely belonging to novel viral families. The prophage species were also found to be widely shared among *R. gnavus* strains, including those belonging to different clades, and there appeared to be no correlation between capsule synthesis and prophage carriage rates. Prophages were also successfully induced from the reference strain, *R. gnavus* ATCC 29149, using mitomycin c, providing experimental validation for the bioinformatic predictions. Together, this work highlights the widespread presence of phages among *R. gnavus* strains, underscoring the need for further research to elucidate their role in shaping *R. gnavus* diversity and facilitating adaptation to the human gut.

B079

Phages manipulate host biofilm in *Bacillus subtilis*

Elvina Smith[1], Robyn Manley[2], Edze Westra[1]

[1]University of Exeter, Penryn, United Kingdom. [2]University of Exeter, Exeter, United Kingdom

Abstract

Parasite manipulation of host social and locomotory behaviour is well-studied within a wide range of Eukaryotic hosts. However, far less is understood about behavioural and social manipulation by parasites in microbial hosts. Biofilms are the predominant mode of bacterial life on the planet and represent a form of bacterial cooperation and social interaction. We investigate how phage-infection alters host behaviour within the social context of bacterial biofilms, using *Bacillus subtilis* and its temperate phages as a model system. We have found that some *Bacillus* infecting temperate phages are able to alter the biofilm structure and motility of their hosts. Infection with phage Phi3t (an SP-Beta phage), significantly reduced host motility and biofilm formation on liquid and solid surfaces, indicating a preference for planktonic growth caused by phage infection.

To determine if this interaction was specific to infection with phage Phi3t, we tested the biofilm forming ability of *B. subtilis* when infected with a range of natural *Bacillus* infecting phages. We found that alteration of biofilm production varied dependent on infection with specific phages. While some phages suppressed host biofilm formation, as was observed during Phi3t infection, other phages promoted it. This indicates phage manipulation and host-phage relationships may vary significantly across phage species. Thus, we show that phages can alter *B. subtilis* biofilm behaviour, and that the nature of this alteration is phage-specific.

B080

Structural and Functional Insights into Gene Transfer Agent DNA Packaging

Rebecca Davies ORCID iD, Paul Fogg ORCID iD, Alfred Antson ORCID iD

University of York, York, United Kingdom

Abstract

Gene transfer agents (GTAs) are virus-like particles produced by some species of Bacteria and Archaea that carry out horizontal gene transfer. GTAs are descended from phages but have evolved various characteristics that prevent virus-like proliferation and spread. These include a lack of DNA replication machinery, little or no preference for packaging of their own genome over their host's, having GTA-coding genes spread across multiple disparate loci, and possessing a capsid with a smaller capacity than the GTA genome. Furthermore, GTA's typically possess a packaging density lower than true viruses, meaning that the capsid is not packaged to full capacity.

Across both true viruses and GTAs, little is known about the terminase proteins responsible for recognising and packaging the DNA. We believe that unique structural features of GTA terminases are responsible for lack of packaging specificity as well as the low packaging density within GTAs, however how they achieve this is unknown. Here, we will show the structures of the GTA large and small terminase subunits determined by cryo-electron microscopy and demonstrate how they interact with each other and with DNA. We will also present the full structure of the *Phaeobacter piscinae* GTA (PpGTA), demonstrating key conserved features of GTAs. Finally, we will present molecular and biochemical data to define the key mechanistic interactions and to understand functional differences between GTAs and phages. Understanding GTA DNA packaging could lead to a simpler model system for studying viral replication and may lead to novel therapeutic targets.

B081

Phage Tricks and Mimicry Magic: Hunting for Hidden Anti-Defence Genes

Samriddhi Gupta, Anna Olina, Aleksei Agapov, Edze Westra, Stineke Van Houte

University of Exeter, Penryn, United Kingdom

Abstract

Bacteriophages, or phages, are viruses that specifically infect bacteria. To survive in environments where bacterial hosts are abundant, bacteria have evolved a wide array of defence systems to recognize and eliminate phages, including CRISPR-Cas systems, restriction-modification systems, and toxin-antitoxin modules. In response, phages have developed anti-defence mechanisms to evade these bacterial defences and successfully establish infections. One particularly intriguing strategy employed by phages is mimicry: they produce proteins that resemble and interfere with components of bacterial defence systems.

Interestingly, "orphan" genes - related to defence systems but not part of a complete defence framework - are frequently observed in temperate and lytic phages. We hypothesize that these orphan genes may use mimicry to imitate bacterial defence components, allowing phages to subvert host defences.

This study employs comprehensive bioinformatic analyses of a large set of Pseudomonas aeruginosa phage genomes to identify and map “orphan” defence genes examining their prevalence, distribution, and genomic context. Following this, we plan experimental validation by expressing candidate orphan genes in P. aeruginosa PAO1 strain equipped with specific defence systems. We will then challenge these strains with phages typically restricted by these defences, assessing if orphan genes disrupt the defence response to facilitate phage infection.

Our research aims to deepen the understanding of phage anti-defence tactics, potentially uncovering novel anti-defence strategies that could inform phage therapy development and shed light on microbial evolutionary dynamics and host-pathogen interactions. We anticipate presenting initial findings from this ongoing investigation at the conference in March.

Session : Urogenital microbes in health and disease

B082

Evaluation of Phytic Acid as a Novel Antimicrobial and Inhibitor of *In Vitro* Urinary Catheter Blockage.

Olivia Ketley, David Williams

Cardiff University, Cardiff, United Kingdom

Abstract

Catheter-associated urinary tract infections from *Proteus mirabilis* crystalline biofilm blockages are the most common nosocomial infection. Research has sought to design antimicrobial catheters, but with little success. Since resistance to conventional antibiotics is rising, the use of novel, naturally-occurring antimicrobials (like phytic acid (IP6)) is appealing. This study aimed to evaluate IP6's antimicrobial efficacy against uropathogens and investigate IP6 delivery methods on urinary catheters (UCs).

IP6's antimicrobial properties were assessed through broth microdilution assays against uropathogens in planktonic and biofilm form. Confocal microscopy (CLSM) was performed on live/dead stained *P. mirabilis* biofilms (from CDC Biofilm Reactors) treated with 1.25%, 2.5% or 5% IP6. Similarly, biofilms from 2.5% IP6 pre-conditioned coupons were investigated for biofilm formation delay. Three IP6 UC delivery methods were explored using *in vitro* bladder models: IP6-soaked UC tips, IP6-filled retention balloon and a IP6 petroleum jelly coating.

Antimicrobial tests established the strains' minimum inhibitory (0.08-0.31%), minimum biocidal (0.31-0.63%) and minimum biofilm eradication concentration (0.63-2.5%) against IP6. CLSM showed even 1.25% IP6 significantly increased dead:live average biomass ratio from 0.467 (untreated) to 0.912. Moreover, biofilms on 2.5% IP6 pre-conditioned coupons had reduced biomass and thickness compared to controls. 2.5% IP6 petroleum jelly coatings significantly increased blockage time by 23.93h.

Overall, IP6's inhibitory effects in microdilution assays were demonstrated at clinically relevant concentrations. CLSM showed IP6 elicits a biocidal effect on *P. mirabilis* biofilms treated post-growth and has antibiofilm properties as a pre-conditioning agent. The 2.5% IP6 petroleum jelly coating indicated potential as an antimicrobial UC coating.

B083

Exploring the Antibacterial and Antioxidant Potential of *Prosopis juliflora*-Derived Copper Nanoparticles Against *Staphylococcus aureus* in Urinary tract infections

Muhammad Zishan Ahmad[1], Syeda Maryam Hussain[1], Ayesha Qaisar[1], Zaib Ur Rehman[1], Muhammad Ali Shah[1], Murtaz Ul Hassan[1], Muhammad Kamran[1], Saif Ur Rehman[1], Aayesha Riaz[1], Muhammad Shoaib[1], Muhammad Usman Naseer[1], Ali Ahmad[2], Nadeem Akhtar[3,1], Zahid Manzoor[1]

[1]PMAS- Arid Agriculture University, Rawalpindi, Pakistan. [2]University of Veterinary & Animal Sciences, Narowal Campus, Lahore, Pakistan. [3]Arsalan Poultry, Rawalpindi, Pakistan

Abstract

Urinary tract infections (UTIs) are a significant public health concern and there are number of microorganisms involved including *Staphylococcus aureus*. Although *S. aureus* is not a major cause of UTIs but resistant pathogen is a potential risk for the population. Traditional antibiotic therapies are facing failure due to increasing prevalence of antibiotic-resistant strains of *S. aureus*. This research investigates the eco-friendly production of copper nanoparticles (CuNPs) with *Prosopis juliflora* as a natural reducing and stabilizing agent. The synthesized CuNPs were characterized using UV-Vis spectroscopy, X-ray diffraction (XRD), energy dispersive X-ray analysis (EDX), Fourier Transform Infrared Spectroscopy (FTIR) and scanning electron microscopy (SEM), which confirmed the generation of stable, well-dispersed nanoparticles. The antibacterial effectiveness of CuNPs was evaluated against *S. aureus*, demonstrating pronounced inhibition zones in disc diffusion assay. The antioxidant capabilities of the CuNPs were assessed using the DPPH test, demonstrating significant free radical scavenging activity. In addition to color shift from light blue to embraled green, further characterization methods verified that the CuNPs were synthesized, that their average size ranged from 10 to 80 nm. CuNPs show antibacterial action when applied to *S. aureus* via disc diffusion. Their results revealed that at 600 ppm, the maximum zone of inhibition was 19.5 ± 0.41 mm, at 400 ppm it was 13 ± 0.82 mm, and at 200 ppm it was 9.1 ± 0.70 mm. CuNPs made from *P. juliflora* are good at killing bacteria, so they could be used as an alternative way to treat UTIs caused by *S. aureus*.

B084

To pee or not to pee? The use of artificial urine media to model bacterial growth and biofilm formation in simulated urinary tract infection.

Ocean E. Clarke ORCID iD[1], Romy A. Dop ORCID iD[1], Tom Hasell ORCID iD[1], Joanne L. Fothergill ORCID iD[1], Daniel R. Neill ORCID iD[2]

[1]University of Liverpool, Liverpool, United Kingdom. [2]University of Dundee, Dundee, United Kingdom

Abstract

The use of artificial urine media (AUM) as an alternative to pooled human urine (PHU) is well established in studies of the urinary tract. Numerous, diverse AUM formulations have been published and utilized throughout research and education, and are often based on historic media formulations or, more recently, urine metabolomic data. Under many circumstances, the use of AUM may prove advantageous over the use of PHU, as solutions can be prepared in large quantities at low cost, and are not subject to the same ethical regulations required for human material use. Additionally, the composition of human urine, *i.e.* hormone and metabolite quantities, varies greatly between individuals, meaning AUM often provides more reproducible data than different batches of PHU.

Despite the extensive use of AUM in research, few studies have validated the use of AUM in the study of urinary tract infections (UTIs) or the urinary microbiome as an accurate replacement for PHU. In this work, we compare the compositions of six well-referenced AUM formulations to PHU, and utilize a panel of prevalent UTI pathogens including *Escherichia coli* to determine which most accurately simulates the urinary tract niche. Collectively, data from this study highlight vast differences in microbial growth and virulence phenotypes between the different AUM formulations assayed. These data will improve understanding of the fundamental biological processes which occur in the urinary tract niche, and aid in providing a more clinically relevant platform for testing novel therapeutics and materials coatings.

B086

In Vitro Modelling of Polymicrobial Infections in the Catheterised Urinary Tract: Biofilm Community Dynamics and the effects of antibiotics ciprofloxacin on polymicrobial communities

Isobel Garratt[1], Maryam Yazdizadeh Ravari[2], Ocean Clarke[2,3], Matthew Wand[4], Mark Sutton[4], Brian Jones[2]

[1]Bath, Bath, United Kingdom. [2]University of Bath, Bath, United Kingdom. [3]University of Liverpool, Liverpool, United Kingdom. [4]UKHSA, Salisbury, United Kingdom

Abstract

Catheter associated urinary tract infections (CAUTI) are highly prevalent hospital acquired infections, causing significant burden on healthcare with many uropathogens implicated in infections being World Health organisation priority pathogens. Up to 75 - 85 % of patients with indwelling catheters have polymicrobial infections (Stickler *et al.,* 2008). There is a gap in knowledge within the current literature with there being few studies on polymicrobial catheter infections, therefore the development of a reproduceable method to model such communities is salient.

This study describes the development of an in vitro model of polymicrobial CAUTIs with common uropathogens (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis* and *Staphylococcus aureu*s), and its application to understanding community dynamics and the effects of antibiotics on biofilm and planktonic communities. Selective medias were developed and validated to facilitate enumeration of specific community members. Biofilm sampling methods were optimised to allow investigation of the biofilm community dynamics. We show polymicrobial communities can be reproducibly modelled using this model and were stable for at least 14 days. The application of ciprofloxacin to polymicrobial communities shows impacts of antibiotics on community dynamics both planktonic and in the biofilm.

This model provides a valuable and robust tool for both basic and applied research in this area, facilitating more robust pre-clinical evaluation of approaches to control biofilm formation, as well as helping to address more fundamental questions relating to biofilm associated infections, the evolution of antimicrobial resistance, and other important traits in these pathogens.

B087

L. iners and P. bivia have a significant effect on HPV E6E7 oncogene expression in NIKS in-vitro cell model

Sophie Stephens ORCID iD, Lauren Roberts, Konstantinos Kechagias, Julian Marchesi, Maria Kyrgiou

Imperial College London, London, United Kingdom

Abstract

Cervical cancer is driven predominantly by persistent infection with high-risk human papillomavirus (hrHPV), yet the role of the cervicovaginal microbiome in HPV persistence and cancer progression is not fully understood. This study investigates the impact of cervicovaginal microbiome metabolites on HPV early promoter activity using the NIKS cell model transfected with HPV18. HPV18 transfected NIKS cells were treated with bacterial conditioned media (CM) from species representative of the cervicovaginal microbiome at concentrations of 2.5%, 5%, and 7.5%. HPV copy number and E6E7 oncogene mRNA expression were measured via qPCR. Results indicated significant increases in E6E7 oncogene expression at lower concentrations of L. iners and P. bivia CM (2.5% and 5%), suggesting these bacteria may enhance HPV oncogene expression. Toxicity in the cells was observed at 7.5%. These findings highlight the potential influence of the cervicovaginal microbiome on HPV-driven oncogenesis, warranting further investigation using 3D cervical organoid models.

B088

In vitro models of urosepsis using synthetic urine stratify E. coli bacteraemia isolates from South West Wales to aid biomarker discovery

Megan Rees ORCID iD[1], Matthew Lewis[1], Heather Chick[1], Michelle Hardman[1], Timothy Ogunrin[1], Mike Simmons[2], Jane Regan[2], Llinos Harris[1], Thomas Wilkinson[1]

[1]Swansea University, Swansea, United Kingdom. [2]Public Health Wales (Microbiology & Health Protection), Glangwili General Hospital, Carmarthen, United Kingdom

Abstract

Sepsis is defined as dysregulation of a host's immune response caused by the spread of a localised infection to the bloodstream and is responsible for 1 in 4 deaths worldwide annually. Urosepsis is caused by a urogenital infection and accounts for between 8-31% of sepsis cases with Escherichia coli (E. coli) being the principle causative organism. The risk of death due to sepsis increases by 7.6% with every 1-hour delay in antibiotics and such delayed diagnosis has been estimated to cost £15.6 billion per annum. Thus, early diagnosis and novel biomarkers are paramount.

To address this issue, blood culture positive E.coli isolates (N=62), were collected from the Hywel Dda University Health Board. Meta data confirmed that 19% were hospital acquired, 44% were urinary based origin of infection, 13% were defined as urosepsis cases, 69% were age ≥65, 40% were male and 13% were fatal. The laboratory phenotypes studied included growth and motility within synthetic fluids.

Exposure of E. coli isolates to increasing concentrations of synthetic urine caused a reduction in isolate growth when compared to LB broth alone. Optimised synthetic urine concentration (80%) was able to group E. coli strains, by virulence characteristics. Motility assays in synthetic urine were consistent with these findings.

These results and isolate collection provide an opportunity to stratify E. coli phenotypes prior to genome wide associated studies (GWAS). This work is likely to identify E. coli genes that can be measured during urinary tract infection that predict invasive translocation to blood to improve clinical intervention.

B089

Using functional genomics to investigate the host-microbe interactions of vaginal *Lactobacillus* species

Phoebe Crossley[1], Sai Ravi Chandra Nori[2], Paul D. Cotter[2,3], Konstantinos Gerasimidis[4], Conor Feehily[1]

[1]School of Infection and Immunity, University of Glasgow, Glasgow, United Kingdom. [2]Teagasc Food Research Centre, Cork, Ireland. [3]APC Microbiome Ireland, University College Cork, Cork, Ireland. [4]School of Medicine, University of Glasgow, Glasgow, United Kingdom

Abstract

Lactobacillus species present in the vaginal microbiome are critical for the maintenance of vaginal health and prevention of pathogen colonisation. The absence of these health-associated lactobacilli has repeatedly been correlated with various adverse gynaecologic and obstetric health outcomes, including an increased risk of preterm birth (PTB). However, the specific mechanisms and host interactions involved in these associations remain elusive. This is predominantly due to limited studies performing in-depth genetic analyses of vaginal *Lactobacillus* species, as well as a lack of available tools for their genetic manipulation.

More recent studies have utilised whole-genome sequencing to identify strain-specific associations in the vaginal microbiome and aid in deciphering these unknown mechanisms. It was recently demonstrated that *Lactobacillus jensenii* strains isolated from vaginal swabs of preterm pregnancies were phylogenetically distinct from those isolated from full-term pregnancies. Additionally, several genetic signatures were identified that distinguished the PTB and full-term birth-associated strains, including genes predicted to be involved in cell wall synthesis, lactate and acetate metabolism, and DNA repair. Preliminary data indicates that these strains have distinct growth phenotypes; however, the genes responsible for this remain unknown. Using homologous recombination based gene knockouts, we are exploring the role of several candidate genes both *in vitro* and in cell culture. These knockouts will help reveal the mechanisms underpinning vaginal lactobacilli-host interactions and determine the implications of these genes in vaginal health and disease.

Session : Enteric Bacteria; Biology, Diversity & Ecology

B093

Microbiome Shifts in Preclinical Alzheimer's Disease: Exploring Gut Dysbiosis in the Presence of Cerebral Amyloidosis

D.M. Sithara Dissanayaka[1,2], Thilini N. Jayasinghe[3], Hamid R. Sohrabi[4,5,2], S.R. Rainey-Smith[4,2], Karen Scott[6], Ralph N. Martins[1,2,5], W.M.A.D. Binosha Fernando[1,2]

[1]Centre of Excellence for Alzheimer's Disease Research & Care, School of Medical and Health Sciences, Edith Cowan University, Joondalup, Australia. [2]Alzheimer's Research Australia, Ralph and Patricia Sarich Neuroscience Research Institute, Nedlands, Australia. [3]The Charles Perkins Centre, The University of Sydney, NSW, Australia. [4]Murdoch University, Murdoch, Australia. [5]Department of Biomedical Sciences, Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, Australia. [6]Microbial Ecology Group, Rowett Institute, University of Aberdeen, Aberdeen, United Kingdom

Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by memory loss and significant behavioral changes. Recent research highlights the critical role of gut dysbiosis in AD pathogenesis, with observed alterations in the gut microbiome among individuals with AD or mild cognitive impairment (MCI). However, the specific variations in microbiota during the preclinical stages of AD, marked by cerebral amyloidosis without cognitive impairment, remain poorly understood. This study aimed to address this knowledge gap.

All study participants (16 CU Aβ+ and 54 CU Aβ-) were selected from highly characterised cohorts and underwent Pittsburg compound B-positron emission tomography. The taxonomic composition of faecal samples was examined through a shotgun metagenomic analysis.

The analysis revealed significant differences in microbial taxa abundances between CU Aβ+ and CU Aβ- groups. At the phylum level, Bacteroidetes exhibited higher abundance in the CU Aβ+ group, contrasting with the predominance of Firmicutes in CU Aβ-. At the species level, *Faecalibacterium prausnitzii* (phylum Firmicutes) and *Bacteroides vulgatus* (phylum Bacteroidetes) showed higher relative abundances in the CU Aβ- group, while *Bacteroides dorei* (phylum Bacteroidetes) was more prevalent in CU Aβ+.

These findings suggest that dysbiosis in Firmicutes and Bacteroides phyla may indicate an imbalance of beneficial and harmful gut bacteria in preclinical AD. Understanding these microbiota shifts could provide insights into early therapeutic targets for managing AD progression, potentially aiding in preclinical AD diagnosis through microbiome analysis. Further research is crucial to uncover the causal relationship between gut microbiota changes and AD onset or progression.

B094

Evaluating green infrastructure's effect on faecal contamination in a suburban drainage system.

Xiaoqi Yu

Newcastle University, Newcastle upon Tyne, United Kingdom

Abstract

As urban suburbs expand, numerous measures have been constructed to alleviate sewage pressure on rivers. Despite separating sewage pipes from surface water drains and placing stormwater retention ponds between surface water drains and receiving waters, several challenges persist, including unauthorized modifications by residents, incorrect pipe connections, and the pollutant contributions by roads and roof runoff. We assessed, for the Great Park suburban area of Newcastle (Northeast England), river pollution by surface water drains and pollution mitigation by intervening stormwater retention ponds. One surface water drain discharges directly into the Ouseburn River, and from 10 sampling events between spring 2023 and summer 2024, mean concentrations of fecal coliforms (FC) and human-host Bacteroides HF183 were significantly higher ($p<0.05$) in the discharge than in the Ouseburn upstream of the drain. During most periods, the fecal coliform load released from this drain (48.7%-89.8%) exceeded the load contributed from the river upstream (10.2%-51%). The reduction capacity of rainwater storage ponds for FC (-3.23% to 38.85), FS (-1.74% to 47.39%), HF183 (-12.20% to 16.36%), and rodA (-0.91% to 23.14%, with extremely low concentrations in spring) showed seasonal trends, with the removal capacity in low-temperature seasons being lower than that in high-temperature seasons. From the Minion sequencing results, the readings of Acinetobacter, Arcobacter, and Faecalibacterium in stormwater retention pond outlet were lower than Great Park east stormwater drain discharge indicating that the stormwater retention pond can help in intercepting pathogenic bacteria.

Key words: River quality, Stormwater drain, Stormwater retention pond.

B095

Is our microbiome friend or foe? Understanding the effect of antibiotics on the gut microbiome.

Yiyun Michelle Fan ORCID iD, Laura de Nies, Mathew Stracy

University of Oxford, Oxford, United Kingdom

Abstract

The human gut microbiome plays an essential role in protecting against colonization and proliferation of pathogens. Simultaneously, the microbiome can serve as a reservoir for opportunistic pathogens to overgrow, potentially leading to disease development under certain conditions. While saving millions of lives, antibiotics are one of the major disturbers of the gut microbiome and affect different individuals to differing degrees. Here, we aim to understand whether and why some people are inherently at high risks of antibiotic-induced perturbations and pathogen overgrowths. We hypothesize that the presence and antibiotic susceptibility of certain bacterial taxa within the baseline microbiota is an important factor underlying individualized responses to antibiotics. By assembling different synthetic microbial communities of common human gut commensals and establishing faecal sample-derived microbial communities, we examined antibiotic-resistant *Escherichia coli* (model pathogen) overgrowth following antibiotic exposure using spot plating and droplet digital PCR. Our findings suggested that synthetic microbial communities with different memberships (and thus different levels of antibiotic susceptibility) supported differing levels of antibiotic-induced pathogen overgrowth. This was further confirmed with complex microbial communities derived from human faecal samples, as faecal-derived communities from different individuals showed varying levels of pathogen growth following antibiotic treatment. Furthermore, we identified *Phocaeicola vulgatus* as a potential keystone species that suppresses *E. coli* growth. By understanding how individual gut microbiota change after antibiotic use, we can implement individualized antibiotic stewardship practices to minimize the collateral damage antibiotics cause to the gut microbiota

B096

Hypervirulent Klebsiella pneumoniae coordinates expression of distinct siderophores in response to different environment cues

To Nguyen[1,2], Sebastian Bruchmann[3], Xenia Kostoulias[1,2,4], Faye C. Morris[1,2], Anton Y. Peleg[1,2,4], Francesca L. Short[1,2]

[1]Infection Program, Monash Biomedicine Discovery Institute, Department of Microbiology, Monash University, Clayton, Melbourne, Australia. [2]Centre to Impact AMR, Monash University, Clayton, Melbourne, Australia. [3]Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom. [4]Department of Infectious Diseases, Alfred Health and Central Clinical School, Monash University, Prahran, Melbourne, Australia

Abstract

Hypervirulent (hvKp) *Klebsiella pneumoniae* is one of two main pathotypes of *Klebsiella pneumoniae*. It is considered a critical public health threat as it can cause community-acquired infection. Siderophores are a major virulence factor of hvKp to acquire iron from the environment. Four different siderophores (aerobactin, yersiniabactin, enterobactin and salmochelin) can be produced in a single hvKp strain and described non-redundant functions. Siderophore expression must be under tight regulatory control to maintain an appropriate amount of intracellular iron to avoid adverse effects to the cells. Though the impact of iron limitation on siderophore expression has been well studied, the contribution of other cues and regulators is not well understood.

In this study, we aimed to understand the regulatory networks governing bacterial iron acquisition in hvKp. Transcriptomics for the hvKp bacterium in 11 infection-relevant stress conditions showed a dynamic response to the stresses by regulating 1.7-43% of the genes in each condition. Interestingly, expression of the siderophore genes was strongly induced by oxidative stress and nutritional downshift whereas they were repressed in low oxygen and other conditions. Growing the bacterium under increasing levels of iron starvation showed a sequential siderophore induction, with enterobactin produced first followed by aerobactin, yersiniabactin and, lastly, salmochelin. *In vivo* expression of the four siderophores revealed that they were found to be differentially expressed in the different host niches.

Overall, our findings support the hypothesis that *K. pneumoniae* uses distinct regulatory mechanisms to control the four siderophores. Further laboratory work will explore the mechanisms underpinning their differential expression.

B097

Understanding the prevalence, genomic epidemiology and transmission dynamics of Campylobacter species in The Gambia

Henry Badji ORCID iD[1], Evangelos Mourkas[2], Abdul Karim Sesay[1], Mehrab Karim[1], Jarra Manneh[1], Binta Faye[1], Ousman Jallow[1], Samba Juma Jallow[1], Madison Goforth[3], Shani UP Ali[3], Bakary Conteh[1], Modou Kandeh[1], Lamin Drammeh[1], Abdoulie F. Jallow[1], Adrian W. Leach[4], Tilly Collins[4], Polina Levontin[4], John D. Mumford[4], Johnson Holt[4], Martin Antonio[1], Ozan Gundogdu[5], Ben Pascoe[3], M. Jahangir Hossain[1], Samuel K. Sheppard[3]

[1]Medical Research Council Unit The Gambia at London School of Hygiene and Tropical Medicine (MRCG at LSHTM), Banjul, Gambia. [2]Uppsala University, Uppsala, Sweden. [3]Oxford University, Oxford, United Kingdom. [4]Imperial College London, London, United Kingdom. [5]London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Campylobacter is a major cause of gastrointestinal disease in Africa, particularly in children under 5 years, with repeated infections resulting in chronic diarrhoeal disease, stunted growth, and mortality. Effective preventive interventions depend on information on quantitative estimates of the burden of disease and infection sources. However, comprehensive data on the reservoirs, epidemiology and transmission mechanisms of *Campylobacter* in Africa is scarce. Genome sequencing and bioinformatics provide a means for explaining disease spread by identifying differences between strains from multiple sources and tracking transmission routes. We aim to evaluate the genomic epidemiology of *Campylobacter* strains from animals, food, environmental sources and both diarrhoeal and diarrhoea-free children from the Genomic Epidemiology and Transmission dynamics of *Campylobacter* species in Africa (GETCampy-Africa, August 2023 – June 2024) study, from the Global Enterics Multicenter Study (GEMS, 2007 – 2012) and Vaccine Impact on Diarrhoea in Africa (VIDA, 2015 – 2018). We will establish an open-access genome database and use genomic analysis techniques to determine the sources of human infection and investigate potential human-to-human transmission. We will employ genomic sequencing to analyse *Campylobacter* strains from GEMS, VIDA and GETCampy-Africa studies to estimate prevalence of infection in children with diarrhoea and to determine the reservoirs of *Campylobacter* infection and identify *Campylobacter* transmission networks. Our study will provide evidence-based and relevant information on the species diversity, prevalence, and transmission mechanisms of *Campylobacter* infection to enable effective local public health and policy interventions and focus efforts towards reducing the burden of diarrhoeal disease in children under 5 years and improving child health.

B098

Investigation of the Campylobacter jejuni Type VI secretion system and its effectors

Molly Webster, Ozan Gundogdu

London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Campylobacter species are the number one cause of human gastroenteritis worldwide, with faecal contamination of animal products being a significant cause of transmission. The ability of *Campylobacter* species to colonise the gastrointestinal tract of their host is a key virulence determinant, with possession of specific sets of genes increasing colonisation ability. The Type VI secretion system (T6SS), made up of 13 core machinery genes, has been shown to provide an advantage in host colonisation. The T6SS is typically located within the ~70 kbp *Campylobacter jejuni* pathogenicity island 1 (CJPI-1) which contains several effector proteins linked to the T6SS. Bioinformatic analysis of the presence and absence of genes within the CJPI-1 was conducted using an 18,764 isolate database of *C. jejuni* and *C. coli* genomes from pubMLST. Approximately 20% of the isolates possessed a T6SS, with T6SS negative isolates still possessing other genes contained in the CJPI-1. The link between the clonal complex, source, and species of isolates possessing the T6SS and those lacking it was assessed. This analysis showed the T6SS presence is more common in *C. jejuni* isolates, isolates from a chicken origin, and isolates belonging to the ST-464 clonal complex. Isolates belonging to the generalist ST-21 and ST-48 clonal complexes were less likely to possess a T6SS.

B100

Crohn's disease adherent, invasive *E. coli* (AIEC) glycolipid receptor interactions with host intestinal epithelium

Hala Layas[1], Massimiliano Stagi ORCID iD[2], Barry Campbell ORCID iD[1]

[1]Infection Biology & Microbiomes, University of Liverpool, Liverpool, United Kingdom.
[2]Biochemistry, Cell & Systems Biology, University of Liverpool, Liverpool, United Kingdom

Abstract

Crohn's disease (CD) is a chronic inflammatory bowel disease affecting millions of people globally. Adherent, Invasive *E. coli* (AIEC) are found in increased numbers associated with the inflamed ileal and colonic mucosae of patients. Previous studies highlight that AIEC adhesins support pathogenicity and suggest that interaction with lipid rafts may facilitate transport across the epithelium. Here, we examined the role of host epithelial cell-surface glycolipids in AIEC interaction with the intestinal epithelium. Two glucosyl-ceramide synthase inhibitors, Genz-123346 and D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), were tested for their action on *E. coli* interaction with the intestinal cell-lines Caco2 and Intestine-407. Following detailed optimisation experiments, a dose of 10 µg/mL over 48h was shown to disrupt major intestinal cell-surface glycosphingolipids GM1, GM3 and GD1 (as assessed by slot blot analysis) without significant cellular cytotoxicity (as assessed by microscopy and cytotoxicity assays). Depletion of cell-surface glycolipids significantly reduced adhesion to, and invasion of, the paradigm ileal CD AIEC isolate LF82, colonic CD AIEC isolates HM605 and HM427, and a P-fimbriated *E. coli* to intestinal cell-lines (all $p<0.01$; Kruskal-Wallis test). Further studies examined AIEC LF82 Long polar fimbriae adhesin (LpfA)-glycolipid interactions using bacterial overlay assays on glycolipids separated by High-Performance Thin-Layer Chromatography. Analysis indicated LF82 interacting with three sialylated gangliosides, with binding lost using LF82Δ*lpfA*, an isogenic mutant strain lacking LpfA, and binding restored using LF82Δ*lpfA*-pBAD24*lpf* (the mutant strain trans-complemented with *lpf* operon). In conclusion, glycosphingolipid receptors, play a role in AIEC-host epithelium interactions. Their presence within cholesterol-rich lipid rafts needs to be further investigated.

B101

Molecular ecology of bacteria in the Rivers Deben and Itchen; from citizen science to metagenomes.

Alyssa Winder[1], Svetlana Gretton ORCID iD[1], Silvia Atanasio[1], Nic Bury ORCID iD[2], Nicholas Tucker ORCID iD[1]

[1]University of Suffolk, Ipswich, United Kingdom. [2]University of Southampton, Southampton, United Kingdom

Abstract

Faecal contamination of our waterways is a hot topic both politically and scientifically. In 2022 >389,000 raw sewage discharges were reported, and The Rivers Trust suggest that only 14% of UK rivers are in good ecological health. We collaborate with citizen science groups who perform traditional water quality testing assays including faecal coliform counts, nitrate and phosphate tests along the River Deben & the Solent.

We have established a workflow to culture isolates from Petrifilm coliform count plates onto MacConkey agar, which shows more strains and taxonomic diversity than those which are counted. Nanopore sequencing reveals the taxonomy of these isolates along with detection of AMR genes and toxins. This analysis detected the presence of 'non-coli' *Escherichia spp.* and *Klebsiella spp.* and >50% of strains carry plasmids. A curated library of 470 strains in 96-well format that facilitates rapid phenotypic screening for biofilm formation, antibiotic resistance and cytotoxicity has been constructed.

Alongside traditional methods, we have also deployed a metagenomic approach at the ports of Felixstowe and Southampton to compare the microbial profiles of these major international ports with more natural estuaries nearby. This approach has allowed us to investigate the contribution of international trade to the microbial diversity (E.g. *Vibrio* Spp.) of our estuaries.

B102

Campylobacter jejuni pathogenesis: modulation of reactive oxygen species production upon interaction with host intestinal epithelial cells

Pranaya Kansakar[1], Geunhye Hong[1], Zahra Omole[1], Nicolae Corcionivoschi[2], Nick Dorrell[1], Brendan Wren[1], Ezra Aksoy[3], Abdi Elmi[1], Ozan Gundogdu[1]

[1]LSHTM, London, United Kingdom. [2]AFBI, Belfast, United Kingdom. [3]QMUL, London, United Kingdom

Abstract

Infectious diarrhoea is a global problem with *Campylobacter* being the most common bacterial cause, accounting for over 80% of human cases. Symptoms typically include bloody diarrhoea, fever and abdominal pains. Despite its importance, the mechanisms by which infection promotes inflammation and disease in humans remain unclear.

ROS production is a well-known antimicrobial defence in innate immunity and now recent work demonstrates its importance in bacterial clearance in epithelial cells. Previous work demonstrates that *Campylobacter* can modulate intracellular and extracellular ROS production in human T84 and Caco-2 cells. *C. jejuni* downregulates the transcription and translation of NOX1 and oxidant defence genes *CAT* and *SOD1*. Furthermore, inhibition of *NOX1* by diphenylene iodonium (DPI) and siRNA reduced the ability to interact, invade, and survive. However, the mechanisms and specific virulence determinants responsible for causing these changes remain unknown.

Here, we use knock-out mutants to test for their ability to invade host cells and activate the NOX pathway of T84 cells, while transcriptional analysis of bacterial and host cells during invasion assays will provide better understandings of genomic changes during infection. Different *C. jejuni* strains and eukaryotic epithelial cells will also be used for cross-validation.

This research will reveal *C. jejuni* virulence determinants that manipulate host ROS production, enhancing our understanding of host-pathogen interactions, and with the hope of developing long-term therapeutic strategies.

B103

Phage-plasmid borne methionine tRNA ligase mediates epidemiologically relevant antimicrobial persistence

P. Malaka De Silva[1], Yi Ling Tam[2], Clare Barker[3,4], Ruizhe Li[1], Leanne Santos[5], Gherard Batisti Biffignandi[1,6], Charlotte Chong[1], Lewis Mason[5,7], Satheesh Nair[3,8], Paolo Ribeca[3,8,7,4], Sion Bayliss[9], Claire Jenkins[3,7], Somenath Bakshi[1], James Hall[5], Lauren Cowley[2], Kate Baker[1,7]

[1]University of Cambridge, Cambridge, United Kingdom. [2]University of Bath, Bath, United Kingdom. [3]United Kingdom Health Security Agency, London, United Kingdom. [4]The James Hutton Institute, Edinburgh, United Kingdom. [5]University of Liverpool, Liverpool, United Kingdom. [6]EMBL - Hinxton, Cambridge, United Kingdom. [7]NIHR HPRU in Gastrointestinal Infections, Liverpool, United Kingdom. [8]NIHR HPRU in Genomics & Enabling Data, Warwick, United Kingdom. [9]University of Bristol, Bristol, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a global public health crisis with few options for control. Therefore, moving to predictive frameworks for identifying emerging bacterial strains capable of rapidly evolving AMR for early intervention is key. Although antimicrobial tolerance and persistence are thought to be precursor phenotypes for AMR, little evidence exists to support their importance in real-world scenarios. We leveraged national genomic surveillance data of diarrhoeal pathogen *Shigella sonnei* (n=3745) to agnostically identify common genetic signatures among lineages convergently evolving toward AMR (n=15) using bacterial genome-wide association. This revealed an association of an AMR trajectory with a highly variable second copy of *metG*, borne on a phage-plasmid we called pWPMR2. Further bioinformatic analyses revealed that pWPMR2 was present across clinical isolates of other enteric pathogens globally, including previous major outbreaks. And, that the mechanism of bearing additional *metG* copies on mobile genetic elements was present across multiple bacterial phyla. Subsequent functional microbiology and experimental evolution studies revealed that expression of additional *metG*, particularly the mutated version on pWPMR2, created a sub population of cells with persister phenotypes that predispose them to the evolution of resistance to third generation cephalosporins. This highlights that the provision of *metG* in trans predisposes bacteria to AMR with real world impacts, likely across a broad range of clinically relevant pathogens. As well as offering a warning sign for emerging AMR lineages, our approach is a timely exemplar of how genomic epidemiology frameworks can rapidly guide functional microbiology studies in the coming era of routine genomic surveillance.

B104

Dissecting the functions of *Campylobacter jejuni* Type VI Secretion System and effectors

Blanca Palmero[1], Zahra Omole[1], Molly Webster[1], Daniel Ken Inaoka ORCID iD[2,3], Ozan Gundogdu ORCID iD[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom. [2]Nagasaki University, Nagasaki, Japan. [3]The University of Tokyo, Tokyo, Japan

Abstract

Campylobacter jejuni is a leading cause of food-borne enterocolitis and ranks among the top four contributors of diarrhoeal disease worldwide. While it poses a significant threat to public health due to increased prevalence and antibiotic resistance, the molecular and cellular mechanisms underlying human enteric disease caused by *C. jejuni* remain poorly understood. Although the recent identification of a Type VI Secretion System (T6SS) in *C. jejuni*, a contact-dependent secretory system responsible for delivering effectors into target cells, has provided insight into its pathogenesis, the specific roles of these effectors are largely unexplored. The presence of a T6SS is associated with greater bacterial virulence, niche adaptation, and survival. The T6SS is found within the *C. jejuni* Pathogenicity Island-1 (CJPI-1), which also encodes nucleases, lipases, effectors, and immunity proteins.

Our reference *C. jejuni* T6SS-positive strain 488 has previously had the intergenic region re-annotated as a pathogenicity island with up- and downstream putative open reading frame product functions described. We have utilised this for effector characterisation (Omole et al., *in preparation*). Here, we provide an updated product function annotation on these putative effectors.

Amino acid sequences from the *C. jejuni* T6SS-positive 488 strain CJPI-1 region were analysed using bioinformatic tools to characterise them with the latest product functions. We utilised sequence homology and motif databases such as PFAM, and PROSITE and AlphaFold outputs to give up-to-date product functions. Our aim is to characterise selected putative effectors to further develop our understanding of the roles of the T6SS in *C. jejuni* pathogenesis and fitness.

B105

Comparing the faecal and caecal microbiomes of broiler chickens in health and intestinal disease conditions.

Marili Vasilogianni ORCID iD[1], Melanie Hay ORCID iD[1], Jonathan Williams ORCID iD[1], Dong Xia ORCID iD[1], Androniki Psifidi ORCID iD[2], Damer Blake ORCID iD[1]

[1]Royal Veterinary College, Department of Pathobiology and Population Sciences, London, United Kingdom. [2]Royal Veterinary College, Department of Clinical Science and Services, London, United Kingdom

Abstract

Broiler chickens are chickens raised for meat production. Intestinal health challenges pose a serious consideration for the welfare and productivity of these animals. The characteristic rapid growth of broiler chickens, which is primarily based on intensive genetic selection, was historically supported by the use of antimicrobials as growth promoters. Since the ban of antimicrobial use for growth promotion, gut health issues have become more prevalent. Currently there is a lack of non-invasive biomarkers for gut health. We hypothesise that associations between the faecal microbiome composition and intestinal health outcomes could permit use of microbial profiling as a non-invasive biomarker of gut health. The most numerous and complex enteric microbial populations are hosted in the caeca, sites of fermentation and volatile fatty acid production. Our study aims to assess and compare the microbial population composition of faecal and caecal samples collected from broiler chickens reared under standard commercial conditions. DNA extracted from faeces and caecal contents of 42 five-week-old broiler chickens with varied intestinal health phenotypes has been characterised through 16S rDNA amplicon and Oxford Nanopore metagenomic sequencing. The faecal and caecal microbial profiles of these chickens are compared to assess the level of caecal microbiome representation in faecal samples and to identify bacterial groups as candidate biomarkers.

B106

Putative lipid transport operon is required for persistence of human gut bacterium Phocaeicola vulgatus in the intestinal tract

Yelizaveta Rassadkina ORCID iD[1], Jayanth Jawahar[1], Rachel Neve[2], Per Malkus[1], Elizabeth Shank[2], Jessica McCann[1], John Rawls[1]

[1]Duke University School of Medicine, Durham, USA. [2]University of Massachusetts Chan Medical School, Worcester, USA

Abstract

Phocaeicola vulgatus (*Pvu*) is a gram-negative anaerobe and one of the most abundant bacterial species in the mammalian intestine. *Pvu* has a rare capability to colonize established gut microbial communities following a single introduction and without antibiotic pre-treatment; however, there is limited information about *Pvu's* ability to colonize and compete within the intestine. Using *in vivo* transposon insertion sequencing (INSeq) we identified genes in *Pvu* strain CL09T03C04 that are differentially required for fitness in germ-free (GF) and conventionally-raised (CR) mice. We identified a gene (*Pvu777*) that appears to be required for *in vivo* fitness in mice with a complex microbiota but dispensable in GF mice. This gene encodes a predicted secreted protein with homology to lipid-binding proteins. *Pvu777* is part of an operon that appears to be unique to the *Phocaeicola* genus, and includes a putative lipid transport protein and DNA binding protein. Strains harboring clean deletions of these genes were subjected to liquid chromatography coupled with mass spectrometry (LCMS) and RNA sequencing (RNA-seq) to identify differences in metabolite production and gene expression. LCMS revealed deficiencies in several lipid species and amino acid derivatives, and RNA-seq suggested that the *Pvu777* gene may be involved in outer membrane function and structure. Ongoing studies seek to elucidate the function of these genes including their roles in the *Pvu in vivo* fitness and competition. The outcomes of this work would advance our understanding of *Pvu* ecology and could be used to control colonization of *Pvu* as a potential probiotic chassis.

B107

D-Serine and D-Tyrosine repress the bacterial genotoxin colibactin

Sofia Sandalli, Emily Addington, Jennifer Hallam, Gillian Douce, Andrew Roe

University of Glasgow, Glasgow, United Kingdom

Abstract

Some intestinal *Escherichia coli* strains, including the well-known probiotic strain EcN, produce a potent genotoxin biosynthesised by the *pks* island. In eukaryotic cells, colibactin induces DNA damage, chromosomal instability, cell-cycle arrest, and is strongly implicated in the development of colorectal cancer. Our results demonstrate the inhibitory effect of multiple amino acids on colibactin production, with particular repression seen with D-Serine and D-Tyrosine. The ability of proteinogenic L-amino acids and corresponding D-enantiomers to repress colibactin production was measured via transcription of the *clbB* gene - necessary for the biosynthesis of the genotoxin - through a *pclbB*:gfp reporter-assay in various types of growth media. Through further *in vitro* and *in cellulo* assays, D-Serine and D-Tyrosine emerged as the most repressive amino acids: in the presence of D-Serine, exposure of plasmid DNA to colibactin resulted in a 45% decrease in levels of cross-linked DNA, whereas in the presence of D-Tyrosine, exposure of plasmid DNA to colibactin resulted in a 60% decrease in levels of cross-linked DNA. We observed that D-Serine furthermore reduces the cytopathic responses typically observed during infection of HeLa cells with *pks+* strains, causing a reduction in host cell senescence of 41%. The capacity of D-Serine and D-Tyrosine to repress colibactin expression was furthermore validated by RNA-seq, and the effectiveness of D-Serine *in vivo* was studied in C57BL/6 mice infected with EcN in an AOM/DSS background. Results showed that D-Serine reduces EcN's probiotic activity *in vivo*, in line with our previous findings showing that D-Serine acts on colibactin to reduce expression.

Session : The fungus among us: confronting antimicrobial resistance in fungi

B110

Detection and Molecular Characterization of Aflatoxin and Ochratoxin Produce Aspergillus Species in Capsicum Spices in Saudi Arabia

Manal Almusa[1], Fatimah Al-Otibi[2], Fahad Alreshoodi[1], Shahad Alsalman[1], Lenah Mukhtar[1], Abdulmohsen Alharbi[1], Zyad Aldosari[1], Mai Alkaleeb[1], Khaloud Alarjani[2], Mustfa Alkhanani[3], Yasser Alrujib[1], Abdulmohsen Aljardan[1], Sulaiman Alajel[1]

[1]Saudi FDA, Riyadh, Saudi Arabia. [2]King Saud University, Riyadh, Saudi Arabia. [3]University of Hafar Albatin, Hafar Albaten, Saudi Arabia

Abstract

Capsicum is the most popular spice used in food flavoring around the globe. However, these spices are prone to mycotoxin contamination. Mycotoxins are natural toxins produced by filamentous fungi including *Aspergillus* species and can pose serious risks to human health. Fungal contamination was assessed in 130 randomly collected samples following ISO 21527-2:2008 standards. Results revealed that 84.6% of the samples exceeded the acceptable fungal count limit (10^2 CFU/g) according to Gulf Cooperation Council (GCC) standards (GSO1016:2015). Surprisingly, packaging had no significant impact on fungal contamination, affecting both packaged (78.5%) and unpackaged (91.6%) samples similarly. The predominant fungal isolates were *Aspergillus* (51.1%), notably *Aspergillus flavus* (38.8%) and *Aspergillus niger* (37.7%). Molecular characterization focused on crucial genes associated with aflatoxin (AF) and ochratoxin (OT) biosynthesis, 14.4% of the isolates exhibited all targeted AF genes, with varying occurrences of individual genes. The mycotoxin analysis, conducted on 34.6% of samples via liquid chromatography-mass spectrometry (LC-MS), detected AFB1 in 28.8% of the samples (0.2-13.8 µg/kg) and OTA in 35.5% (6.87-59.00 µg/kg). The findings emphasize the need for regulations governing the storage, handling, and packaging of spices in Saudi Arabia to combat toxigenic fungal contamination and mycotoxin presence. This study stands out as the first in Saudi Arabia to focus on *Aspergillus* in *Capsicum* products and contributes to food safety knowledge in the region.

B111

Proteomic Analysis of the Response of Candida albicans to Novel Silver Based Antifungal Compounds

Daryl Reidy, clara evans, denise Rooney, frances Heaney, Kevin Kavanagh

maynooth university, Maynooth, Ireland

Abstract

Candida albicans is an opportunistic human pathogenic yeast which can cause a range of infections including candidosis, which affects 75% of women throughout their lives. Although there are limited treatment options available, there is a growing concern around the use of these antifungals which can display high levels of toxicity. There is also a growing concern around the emergence of antifungal resistance which has been described in many pathogenic fungi, including *C. albicans.* There is historical precedence for the use of silver as an effective antimicrobial agent, more recently, there has been interest in silver(I) complexes containing phenanthroline ligands. Based on this, we have synthesised a suite of silver complexes to investigate this activity. Ag-PPO is a silver-based compound, comprised of a single silver cation bound to two identical phenanthroline-oxazine ligands each bearing a propyl side chain, structure 1. Ag-PPO displays very strong antifungal activity. A label free quantitative proteomic analysis was conducted on *C. albicans* cell exposed to Ag-PPO to determine a potential mechanism of action. This analysis identified potential disruption of ribosome biosynthesis, and cellular respiration indicating potent antifungal activity which could bypass known antifungal resistance mechanisms. Proteins such as Acyl-CoA-binding protein and NADH dehydrogenase 1-β-subcomplex subunit 7, which are heavily involved in cellular respiration, were significantly decreased (-60.8, and -71.9-fold) in the treated group. Statistical analysis revealed no proteins which were significantly increased in abundance, suggesting a total metabolic shutdown.

B112

Elucidating the molecular mechanisms of novel antimicrobial peptides in Candida infection control

Ahmad Alhanich, Stephen Cochrane, Linda Oyama

Queen's University Belfast, Belfast, United Kingdom

Abstract

Fungal infections are becoming more difficult to treat owing to the increased incidence of multi/pan-resistant fungal pathogens like *Candida auris* and *Candida glabrata*.

Candida auris was discovered in 2009 and has since spread globally causing nosocomial infections of the bloodstream, heart, nervous system, and bones with a mortality ranging from 29% to 53%. Between 87% to 100% of all *Candida auris* strains are resistant to Fluconazole, and 35% to Amphotericin-B, with variable resistance rates to other Azoles and Echinocandins.

There is therefore an urgent need to develop alternative and more effective therapies, especially for the multidrug resistant critical priority pathogen *Candida auris*. In this study, we aim to investigate antimicrobial peptides (AMPs) as a potential solution to the antifungal resistance challenge, as they are known to be fast acting exhibiting antimicrobial activity via their membrane disrupting or metabolic pathway disrupting capabilities, with relatively slower likelihood of resistance arising.

Based on previous in silico tools developed for identification of novel peptides candidates, we will identify and engineer antimicrobial peptides with high selectivity that are both effective against *Candida auris* and have a high therapeutic index and no toxicity to human cells. We have already identified some AMPs with activity against *Candida auris* and further understanding of their mechanisms of actions and interaction with pathogen and host cells is key for our fight against mycotic AMR.

Session : Environmental, Applied & Industrial Microbiology Forum

B114

Metabolomic profiling and pharmacological Potential of Marine- derived Actinomycetes *Nocardiopsis alba* from the Coastal Regions of Arabian Sea

Deepa Mathew P, Dr. V. Robin Perinba Smith

Department of Zoology, Scott Christian College, Nagercoil, Kanyakumari District, Tamilnadu,, India

Abstract

The emergence of multidrug-resistant uropathogens poses an imminent danger to humankind. Infections with multidrug-resistant bacteria are hard to treat. The marine actinomycetes are a rich source for novel biomolecules. The present study, 'Metabolomic Profiling and Evaluation of Antibacterial Potential of Marine-derived Actinomycetes *Nocardiopsis alba* from the Coastal Regions of the Arabian Sea,' aims to prove marine actinomycetes have some bioactive secondary metabolites that are antagonistic to multidrug-resistant uropathogens. . The in vitro antibacterial activity of marine actinomycetes isolates was evaluated against 5 prominent multidrug-resistant uropathogens by agar-well diffusion assay and disc diffusion assay. The ethyl acetate extracts of selected actinomycetes isolates showed excellent antibacterial activity against all the tested pathogens, with zones of inhibition ranging from 12 to 18 mm in disc diffusion and 20–24 mm in well diffusion assay. The result was consistent on both well diffusion and disc diffusion. This study concludes that the ethyl acetate extract of the marine sediment-derived actinomycetes can be taken forward as a promising candidate against multidrug-resistant bacterial pathogens. The initial in vitro experiment confirmed the efficacy of marine actinomycetes secondary metabolites as natural antimicrobial compounds, thereby suggesting the possibility of employing them in drugs for the treatment of infectious diseases. The marine actinomycetes strain *Nocardiopsis alba* (SD9) with antagonistic activity against selected uropathogens was selected for the metabolomics studies. Metabolic profiling was done using the UPLC-MS/MS method. Non-targeted LC-MS/MS revealed high metabolite diversity, including several known metabolites, such as cyclopamine, metabutethamine, and amifloxacin.

Keywords: Marine actinomycetes, Secondary metabolites, Uropathogens, metabolomics

B115

Molecular Characterization of Fungi Associated with Pod Rot of the African Locust Beans (Parkia biglobosa)

Adebola Azeez Lateef, Medinat Adetutu AbdulKareem, Kamoldeen Abiodun Ajijolakewu, AbdulKabeer Omeiza AbdulMalik

Department of Plant Biology, University of Ilorin, Ilorin, Nigeria

Abstract

Parkia biglobosa is the African locust bean, native to West Africa and majorly used in the production of "Iru" a local soup sweetener in various African delicacies as alternative to monosodium glutamate (MSG) which detrimental long-term effects in the body. However, pod rot disease of the *P. biglobosa* pods leads to considerable losses in "Iru" production. This study was therefore carried out to identify the causal pathogen associated with *Parkia biglobosa* pod rot disease using sanger sequencing. 12 Diseased pods of *P. biglobosa* were collected and processed for isolation of the fungal pathogen based on their morphological characteristics. Genomic DNA was extracted from the isolate, amplified with primers ITS5/ITS4 and Sanger sequencing of the internal transcribed spacer (ITS) region of the pathogen was used to identify the fungal pathogen with the molecular analysis software Molecular Evolutionary Genetics Analysis (MEGA). Pathogenicity test was carried out to confirm the potential of the isolated pathogen to cause the implied disease. The pathogen was identified to be *Pseudofusicoccum violaceum* with 562 bp and 99-100 % sequence identity with GenBank database. Koch's postulates confirmed the pathogenicity of the isolate on *P. biglobosa* pods. This study constitutes the first report of *Pseudofusicoccum violaceum* as the causal pathogen of *P. biglobosa* pod rot in Nigeria. The findings from this study provide information on the ecological significance of *Pseudofusicoccum violaceum* as pathogen on *P. biglobosa* and possible control strategies of the disease caused.

B116

Using bar-seq to understand the genome dynamics of *Streptomyces clavuligerus* during industrial fermentations

James Croxford[1], Steven Kendrew[2], Benjamin Huckle[2], Iain Hunter[1], Paul Hoskisson[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]GSK, Worthing, United Kingdom

Abstract

Endemic antimicrobial resistance underscores the urgent need for new antibiotics and a deeper understanding of antimicrobial production by microorganisms. Clavulanic acid (CA), a β-lactamase inhibitor co-formulated with amoxicillin to boost efficacy, is produced industrially by *Streptomyces clavuligerus*. Decades of empirical strain improvement, have increased CA yields potentially at the cost of genetic stability. Genome sequencing reveals a highly dynamic genome in these strains, which can impact on CA production during fermentation. We have repurposed barcode sequencing to help link the genotype of *S. clavuligerus* to its phenotype in industrial fermentations, enabling us to track strain abundance over time and identify the emergence of dominant strains. Our findings indicate that fermentation conditions, including media composition and scale, influence strain dynamics. Notably, we observed a significant shift in the abundance of the four indigenous plasmids of *S. clavuligerus* in response to fermentation conditions. The plasmid pSCL3 is often lost, which appears to impact hyphal branching—a phenotype that warrants further investigation. This study advances our understanding of the genetic and phenotypic dynamics of *S. clavuligerus* during fermentation, offering insights for optimising CA production in industrial settings.

B117

Enhanced Nutritional Profile of Canola Meal via Fermentation with Aspergillus niger and Trichoderma reesei for feed application

Ahmad Alhomodi[1], Majid Al-Zahrani[2], Yousif S Adam[1]

[1]Najran University, Najran, Saudi Arabia. [2]King Abdulaziz University, Rabigh, Saudi Arabia

Abstract

The presence of antinutritional factors (ANFs) in canola meal limits its application as a feed ingredient despite its high protein content. This study aimed to enhance the nutritional profile of canola meal through solid-state fermentation using *Aspergillus niger* and *Trichoderma reesei*. Fermentation significantly increased the protein content, with *A. niger* and *T. reesei* achieving 45.07% and 44.06%, respectively, compared to 40.12% in the control. Crude fiber content slightly decreased in *A. niger* by 2.6% compared to control while *T. reesei* showed no change. Notably, fermentation with *A. niger* substantially reduced phytic acid (0.7 g/100g) compared to the control (2.7 g/100g) whereas *T. reesei* exhibited a less reduction (3.19 g/100g). Total phenolic content increased in both treatments, with *A. niger* (17.6 mg/g) and *T. reesei* (15.55 mg/g) compared to the control (13.6 mg/g). Glucose levels decreased in *A. niger* (0.06 mg/ml) and *T. reesei* (1.81 mg/ml) compared to the control (4.02 mg/ml). These results suggest that fermentation with *A. niger* is particularly effective in reducing ANFs, while both fungi enhance the protein content of canola meal for feed application

B118

Microbial Inactivation in Wastewater Treatment Using Dielectric Barrier Discharge Cold Plasma

José Gonçalves ORCID iD[1], Tom Koritnik[2], Davor Krzisnik[3], Jure Zigon[3]

[1]NOVA UNiversity Lisbon, Caparica, Portugal. [2]National Laboratory of Health, Environment and Food, Ljubljana, Slovenia. [3]University of Ljubljana, Ljubljana, Slovenia

Abstract

Water scarcity, driven by climate change, population growth, and pollution, is a critical global issue, particularly affecting water-stressed regions. Microbial contamination of water resources further exacerbates this challenge, increasing the risk of waterborne diseases. To address this, novel water treatment technologies are needed to ensure microbial safety in recycled water. Cold plasma (CP) technology, specifically dielectric barrier discharge (DBD) CP, offers a promising solution for microbial inactivation in wastewater. CP generates a mixture of reactive oxygen and nitrogen species (ROS and RNS), which can effectively disrupt microbial cells. This study focuses on evaluating the efficiency of a DBD CP system for inactivating gram-positive and gram-negative bacteria in water samples. Escherichia coli (DH5α) and Bacillus subtilis (L12) were used as model organisms representing common pathogens found in contaminated water. The results demonstrated rapid bacterial inactivation, with significant reductions in viable cell counts after just a few seconds of plasma exposure. Complete inactivation was achieved within 15 seconds for both strains, showcasing the potential of DBD CP for microbial control. Additionally, the system's scalability and low operational cost make it suitable for widespread application in wastewater treatment facilities. This research highlights the importance of advancing microbiological water treatment methods to combat the growing threat of waterborne diseases. Further investigations will expand the scope of this study by exploring CP's effects on biofilms and antimicrobial resistance genes, contributing to the development of more comprehensive wastewater treatment technologies.

B119

Potentials of lactic acid bacteria associated with fermentation of maize for production of ogi - a local weaning food in Nigeria

Ejikeme Nwachukwu ORCID iD

Michael Okpara University of Agriculture Nigeria, Umuahia, Nigeria

Abstract

Statement of the Problem: In Nigeria, ogi is a fermented cereal food. Ogi is a local food for weaning infants especially by low income earners that cannot afford imported infant foods. The process of production of ogi has some challenges related to microbial pathogens which cause childhood diarrhoea. There is currently no conditions and practices that preserve the quality of ogi to prevent contamination and food-borne illnesses. This study aimed to improve safety of ogi production for human consumption.

Methodology: The modified indigenous traditional method for processing maize into ogi was adopted. The characteristics and potentials of lactic acid bacteria were determined. Lactic acid bacteria associated during the traditional fermentation of ogi were identified by molecular methods. The bio preservation potentials and antimicrobial activity of the lactic acid bacteria against food spoilage microorganisms were determined. The probiotic potentials of lactic acid bacteria were evaluated.

Results: The isolates were identified on the basis of genotypic characteristics as Lactobacillus amylolyticus strain L6, Lactobacillus plantarum strain ci-4w and Lactobacillus sakei strain MLSI. These isolates indicated antimicrobial activity against spoilage organisms (Pseudomonas aeruginosa, Enterobacter aerogenes and Bacillus cereus). They showed probiotic properties.

Conclusion & Significance: This research provides health benefits as it evaluates probiotics properties and potential to enhance the shelf-life and safety of ogi through assessing the antimicrobial activity against food spoilage organisms and bio preservation potency of lactic acid bacteria. The lactic acid bacteria isolated can be used as potential starter cultures with predictable characteristics in the production of ogi.

B120

PROBIOTIC POTENTIAL OF RIBOFLAVIN-OVERPRODUCING *Bacillus subtilis* ACU-I163MR AND ACU-I11MR, ISOLATED FROM FERMENTED AFRICAN LOCUST BEANS

Theresa Awotundun ORCID iD, Afolake Olanbiwoninu

Ajayi Crowther University, Oyo, Nigeria

Abstract

Riboflavin (Vitamin B_2) is a water-soluble compound that plays an important role in multiple cellular functions. This study evaluates the probiotic potential of riboflavin-overproducing *Bacillus subtilis* strains isolated from fermented African locust beans. After strain improvement, *Bacillus subtilis* ACU-I11MR and ACU-I163MR were selected due to their higher riboflavin production (0.01905±0.0005 mg/L to 0.0259±0.0077 mg/L and 0.0195±0.0054 mg/L to 0.0267±0.0013 mg/L, respectively). Their safety was confirmed through haemolytic assay, antibiotic susceptibility tests, and the absence of gelatinase and biogenic amine activity. Probiotic potential was assessed via *in vitro* assays including resistance to low pH, bile salts, phenol, temperature, and NaCl; auto-aggregation; cell hydrophobicity; biofilm formation; antibacterial activity; enzyme and exopolysaccharides production. Both strains were non-haemolytic, negative for gelatinase and biogenic amine activity. They showed significant viability at pH 2 (survival 85.05; 87.09%), 1% bile salts (survival 88.82; 87.64%), and 0.5% phenol (survival 48.80; 59.52%) respectively. ACU-I11MR was susceptible to 9 out of 12 antibiotics, while ACU-I163MR was 100% susceptible. The strains demonstrated strong cell surface adhesion and auto-aggregation, and inhibited several pathogenic bacteria. They produce amylase, protease, and exopolysaccharide, and thrived under various temperature and NaCl conditions. *Bacillus subtilis* ACU-I163MR, showing superior probiotic potential, could be a promising candidate for developing riboflavin-enriched *Bacillus*-fermented functional foods.

B121

Antimicrobial and Antioxidant Activities of Ganoderma lucidum mediated Silver, Gold, and Silver-Gold Alloy Nanoparticles

Elijah Adegoke ADEBAYO ORCID iD[1,2], O.O Oyeleke[3], N.A Ajisope[4], T.A Kilani[4], A Lateef[4], M.A Azeez[4], T.A Yekeen[4], L.S Beukes[5], N Matyumza[6], E.B Gueguim-Kana[5]

[1]Ladoke Akintola University of Technology, Ogbomoso, Ogbomoso, Nigeria. [2]Ajayi Crowther University, Oyo, Oyo, Nigeria. [3]LadokeAkintola University of Technology, Ogbomoso, Nigeria, Ogbomoso, Nigeria. [4]Ladoke Akintola University of Technology, Ogbomoso, Nigeria, Ogbomoso, Nigeria. [5]University of KwaZulu-Natal, Private Bag X01, Scottsville,, Scottsville, Pietermaritzburg, South Africa. [6]University of KwaZulu-Natal, Private Bag X01, Scottsville,, Pietermaritzburg, South Africa

Abstract

Oxidative stress and threats raised by antibiotic-resistant bacteria infections in recent years have increased significantly. Strong cytotoxic effects on humans by some synthetic antioxidants and high antibiotic-resistant bacteria have been reported, hence, there is a need for an urgent alternative. *Ganoderma lucidum* was employed in this study to synthesize silver, gold, and silver-gold nanoparticles for their antioxidants and antimicrobial. Synthesized nanoparticles were characterized and evaluated for their antibacterial, antifungal, and antioxidant activities. The surface plasmon resonance UV-Vis spectra for GL-AgNPs, GL-AuNPs, and GL-Ag-AuNPs are 430, 550, and 540 nm respectively. The significant FTIR peaks 3227 cm^{-1} (GL-AgNPs), 3437 cm^{-1} (GL-AuNPs), 3437 cm^{-1} (GL-Ag-AuNPs) indicate the amines which could be responsible for the reduction of the metal ions, capping and stabilization of the nanoparticles. The particles were predominantly spherical with sizes ranging from 11.06 - 51.12 nm (GL-AgNPs), 9.01 - 30.56 nm (GL-AuNPs), 9.18 - 45.79 nm (GL-Ag-AuNPs), and the SAED showed that they are crystalline. Highest antibacterial activities (21.33 mm) were obtained at 100 µg/mL from GL-AgNPs, while GL-AuNPs gave the highest (83.75%) of the antifungal at 150 µg/ml. The DPPH-radical scavenging, nitric Oxide Scavenging, total phenolics, and flavonoid content revealed a good antioxidant potential of the synthesized nanoparticles in comparison with the *G. lucidum* extract and standard (BHA and ascorbic acid). Results showed dose-dependent activities at tested concentrations (20 – 100 µg/mL). This study has established the potential of *G. lucidum* to synthesize AgNPs, AuNPs, and Ag-AuNPs, and the efficacy of synthesized nanoparticles as antimicrobials and antioxidants.

B122

Structural and functional alterations in human gut microbiome due to long-term arsenic exposure

Soma Ghosh ORCID iD[1,2], Shankha Nath[3], Subhamoy Bhowmick[2], Arijit Chakraborty[2], Kunal Majumdar[4], Souvik Mukherjee[3], Sreemanta Pramanik[2]

[1]Adamas University, Kolkata, India. [2]CSIR-National Environmental Engineering Research Institute, Nagpur, India. [3]BRICS-National Institute of Biomedical Genomics, Kalyani, India. [4]KPC Medical College and Hospital, Kolkata, India

Abstract

Human gut microflora, known as 'second human genome' comprises 500 – 1000 species, the diversity and richness of which is found to be perturbed upon exposure to various environmental stress factors. Arsenic, a notorious Type I carcinogen found in groundwater and diet of West Bengal, India is expected to perturb the gut microbiome of the exposed population. 16S rRNA amplicon sequencing of faecal metagenomic DNA obtained from subjects with skin lesion (case; n=49), and no skin lesion (control; n=48) from As endemic (Nadia) zone and healthy control (n=48) from non-endemic (Paschim Medinipore) zone was performed. Bioinformatic analysis using QIIME, PICRUSt analysis to decipher the predicted gene expression and network analysis using Cytoscape and NETSHIFT were performed to assess the impact of long-term arsenic exposure on human gut microbiome. Among the 26 phyla detected, significant perturbation was noted in Bacteroidetes, Actinobacteria, Proteobacteria, and Firmicutes in exposed group. Notable reduction of α-diversity due to arsenic exposure indicated a reduced species richness with altered abundance. β- diversity analysis revealed prominent inter-individual differences in subjects from exposed and unexposed areas. PICRUSt analysis predicted significant upregulation (pT-test< 0.05) for gene families belonging to metabolism of carbohydrate, amino acid, nucleotide and lipids along with fermentation and secondary metabolite or vitamin synthesis pathways. A major disruption in the bacterial interactions was noted with 99 nodes in unexposed while only 22 and 17 nodes in exposed groups, respectively. A correction in the gut microbiome composition through probiotic formulation may alleviate the toxic effect of As exposure.

B123

Studies on the inhibition of *Listeria monocytogenes* in different cooked ham formulations

Artemis Zavitsanou[1,2], Grace Kennedy[2], Paul Whyte[2], Declan Bolton[1]

[1]Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland. [2]University College Dublin, Belfield, Dublin 4, Ireland

Abstract

Concerns about nitrosamines and an increased risk of cancer have motivated research on finding novel ham formulations with reduced nitrite and using alternative compounds. The objective of this study was to determine if *Listeria monocytogenes* growth could be prevented in cooked hams formulated with sodium nitrite, sodium ascorbate and sodium chloride. In addition to the control, 3 different ham formulations ([1] 120ppm sodium nitrite, 1000ppm sodium ascorbate and 2.5% (w/w) sodium chloride; [2] 1000ppm sodium ascorbate and 2.5% (w/w) sodium chloride, and [3] 1000ppm sodium ascorbate and 4% (w/w) sodium chloride) were produced, inoculated with *L. monocytogenes* (ATCC 35152) and incubated aerobically at both 4°C and 20°C. At 4°C, the bacteria grew in all tested hams with significantly ($p < 0.05$) higher growth in ham formulations 1 and 2 as compared to 3 (i.e. control > ham 1 = ham 2 > ham 3). At 20°C, the pattern of growth was control = ham 1 = ham 2 = ham 3. It was concluded that none of the formulations prevented the growth of *L. monocytogenes* at 4°C or 20°C, and although the combination of 4°C and formula 3 demonstrated enhanced inhibition, this was not sufficient to prevent pathogen growth and assure food safety.

B124

Partnership Selection Dynamics in Clover-Rhizobium Symbiosis

Samia Riaz

Clark University, Worcester, USA

Abstract

The rhizosphere is a complicated network where plant-microbe interactions significantly boost biodiversity and ecosystem productivity. My research explores the symbiotic relationship between Trifolium barbigerum (Bearded Clover) and rhizobia, aiming to understand how clover identifies the most beneficial microbial partners to optimize nodulation and nitrogen fixation, vital processes for soil health and plant growth. We hypothesize that Trifolium barbigerum selectively forms more nodules with beneficial rhizobia strains, shows a preference for known strains across generational shifts, and adjusts its fidelity based on the efficiency of the rhizobia strains. To test these hypotheses, experiments will be conducted under a controlled environment to assess patterns of nodulation and analyze the mechanisms involved in microbial selection and recognition. Statistical analysis will explore the correlation between strain efficiency and plant fidelity. The goal of my work is to advance our understanding of plant-microbe dynamics, enhancing agricultural sustainability, and contributing to food security and environmental resilience. The findings would not only elucidate the mechanisms of symbiotic partner selection but also address broader ecological questions regarding resource allocation and evolutionary pressures in mutualistic relationships

B125

An Assessment of Dip-slides for Liquid Bioburden Monitoring.

Sophie Hibberd, James Redfern, Edward Randviir

Manchester Metropolitan University, Manchester, United Kingdom

Abstract

Dip-Slides were developed in 1965 for urinary tract infection diagnosis without laboratory equipment or expertise and, through immediate inoculation, to eliminate the bias caused by bacterial multiplication during transportation, providing simple microbiological testing applicable to various industries. The caveat to their simplicity is that quantification is restricted to the nearest estimated $\log^{10}\pm1$ CFU/ml as bacterial growth is compared to a colony density chart. This study demonstrates key limitations of dip-slide testing, by assessing an alternative quantification method (counting colonies), and investigating the impact of immersion time, which varies between studies, on the contamination detected. Bacterial suspensions of E.coli, S.aureus, P.aeruginosa, K.pneumoniae, A.baumannii were used, and bacterial concentrations measured by spread plate and dip-slide simultaneously.

Significantly higher bioburden was detected after five minute dip-slide immersion compared to shorter durations, however the result seen after one minute was closest to that calculated using spread plates. Agar type also impacted results; MacConkey agar detected higher contamination than CLED agar on the same dip-slide. A weak correlation (R=0.3021) was found between spread plates and colony counts from dip-slides, implying this is not a suitable quantification method, however significant positive correlation was seen when pure cultures with concentrations between 10^2 - 10^8 CFU/ml were assessed using the colony density chart. At concentrations below 10^5 however, only E.coli demonstrated significant correlation, implying the dip-slide is not suitable for applications with contamination below 10^5 CFU/ml. Systems still reliant upon dip-slides should consider MicroVal approved alternatives with similar ease of use (eg. Petrifilm and CompactDry).

B126

Survival Beyond The Gut: The Evolution of Aerotolerance in *Campylobacter jejuni*

Shani Ali ORCID iD[1], Evangelos Mourkas ORCID iD[2], Ben Pascoe ORCID iD[3], Aidan Taylor ORCID iD[4], Samuel Sheppard ORCID iD[3], Tiffany Taylor ORCID iD[1]

[1]University of Bath, Bath, United Kingdom. [2]Uppsala University, Uppsala, Sweden. [3]University of Oxford, Oxford, United Kingdom. [4]University of Reading, Reading, United Kingdom

Abstract

One of the most common causes of diarhoeal disease is *Campylobacter jejuni* (*C. jejuni*). As a microaerophilic bacterium, *C. jejuni* thrives in low oxygen environments, primarily adapting to the gastrointestinal tracts of birds. However, isolates have been identified from diverse sources, including water, poultry production lines, and dairy products, suggest potential adaptations to survive higher oxygen concentrations, termed aerotolerance. Aerotolerance could enhance transmission by prolonging environmental survival. Despite of its fastidious nature, *C. jejuni's* adaptability to atmospheric changes raises questions about its response to environmental stressors within transmission networks. To delve into the dynamics and mechanisms of *C. jejuni's* environmental survival, we employed experimental evolution. Four isolates underwent serial passages and incubation in shaking culture to induce aerobic stress. Changes in colony forming units under both ideal microaerophilic conditions and aerobic stress were monitored throughout the experiment. The evolved lines were sequenced and compared to the original ancestral strains and controls, facilitating the identification of candidate genes potentially involved in stress tolerance. Minimum inhibitory concentrations of antibiotics including ciprofloxacin and fluoroquinolones will be determined in ancestral and evolved lines to investigate cross tolerance across stressors. Our study aims to shed light on the adaptability of *C. jejuni* to environmental oxygen levels, highlighting possible oxygen tolerance. We hope to identify candidate genes to allow insight into the molecular mechanisms allowing survival along *C. jejuni* transmission networks. Further investigation and research of identified genes to understand their role in survival may inform more targeted interventions and control measure to reduce *C.jejuni* infection.

B127

The Role of the Soil Microbiome in Carbon Sequestration and Capture in Soils Amended with Mineral Wastes

Samuel Marron ORCID iD[1,2], Angela Sherry[1], Kevin Stott[1], Miranda Prendergast-Miller[1], Mark Goddard[1]

[1]Northumbria University, Newcastle Upon Tyne, United Kingdom. [2]Newcastle University, Newcastle Upon Tyne, United Kingdom

Abstract

Since the turn of the century, a new term has been increasingly used to define a new planetary epoch 'The Anthropocene'. The prominent markers of the Anthropocene include climate change, rising global temperatures, and the changing chemical compositions of soils and oceans. The IPCC predicts overshooting 1.5 °C, even for a short period, will lead to severe, and irreversible impacts. To remain within 1.5 °C society must not only shift to a net-zero energy system but also implement carbon removal methods, such as carbon sequestration. Carbon sequestration is mediated by many microorganisms that induce carbonate precipitation through various metabolic processes including ureolysis, sulphate reduction, or photosynthesis. A new enhanced form of carbon sequestration has been proposed, a nature-based carbon capture technique called enhanced weathering, which involves spreading finely ground silicate rock upon the land to accelerate natural weathering. Enhanced weathering has the potential to offset carbon emissions, it also improves soil health and fertility. Despite this, there is a limited understanding of the full diversity of carbonate-precipitating microbes within the soil microbiome and the microbial interactions behind inorganic carbon capture within soils are poorly defined. Our goal is to understand the microbial diversity of mineral-waste amended soils, determine crucial functional genes and metabolic pathways through DNA-based molecular methods to elucidate the role the microbiome (bacteria, fungi, and viruses) plays in carbonate-precipitation in soils. Knowledge provided from this project will contribute to a greater understanding of the role the soil microbiome performs in carbon sequestration, and better land management practices.

B128

Tracking the Evolution of a Marine Microbial Community under Prolonged Benzene Exposure

Amy Rose Birss[1,2], Jagroop Pandhal[1], Niranjan Nagarajan[2,3]

[1]University of Sheffield, Sheffield, United Kingdom. [2]A* Genome Institute, Singapore, Singapore. [3]National University of Singapore, Singapore, Singapore

Abstract

Marine environments contaminated with volatile organic compounds (VOCs) require efficient bioremediation to minimise the negative impacts on human health and the environment. Microalgae and bacteria consortia can function in tandem to remediate contaminants such as VOCs. This research explores the genotypic and phenotypic adaptations of marine microbial consortia under selective benzene exposure and long-term cultivation to improve VOC degradation and symbiotic interactions. Using multi-omic and bioinformatics tools, including GraphMap, we characterize community-wide genetic, phenotypic and mutational changes driven by prolonged benzene exposure.

Adaptive laboratory evolution (ALE) is applied to the diverse marine community, aiming to strengthen the symbiotic relationships within the consortium and enhance resilience and cooperative functionality under VOC stress. Metagenomic analyses are used to characterise the evolution of the community throughout ALE, providing insight into the mechanisms of microbial adaptation to VOCs. Both shotgun and amplicon sequencing are used to explore the diversity of the community.

B129

The potential of soil anaerobic microbial communities for Polylactic acid (PLA) bioplastic degradation.

Chatuphon Siamphan

Newcastle University, Newcastle upon Tyne, United Kingdom

Abstract

High demand (60% of global biodegradable bioplastic production) for polylactic acid (PLA), a biopolymer made by biobased material from microbes and plant products, as a sustainable alternative to plastic means understanding its degradation under different environmental conditions is essential. The simulation experiment investigated bioplastic waste degradation under anaerobic conditions using a microcosm to understand the microbial communities involved in the PLA degradation process. The accumulation of greenhouse gases, including carbon dioxide (CO_2) and methane (CH_4) in the headspace, indicated that PLA had degraded and was utilised in microbial activity under mesophilic (30°C) and thermophilic (50°C) conditions after 150 days. The results show a significant difference in gas production between soil and sediment in this study, which substantially affected the degradation of PLA, especially under thermophilic conditions. Total CO_2 and CH_4 production under mesophilic conditions were 168.5 and 93.8 mmol, respectively, which were lower than the production under thermophilic conditions at 261.4 and 205.5 mmol. Furthermore, the 16S rRNA analysis revealed differences in the composition of the microbial community, depending on the temperature conditions and soil locations.These findings lead to an understanding of how to optimise PLA biodegradation processes, which contributes to a sustainable waste management strategy.

B130

The *E. coli lite* strains: a new expression platform for the production of toxic membrane proteins.

Alexandra Trigg[1], Parisa Pordelkhaki[2], Stephen Busby ORCID iD[3], Tim Overton ORCID iD[2], Douglas Browning ORCID iD[1]

[1]School of Biosciences, Aston University, Birmingham, United Kingdom. [2]School of Chemical Engineering, University of Birmingham, Birmingham, United Kingdom. [3]School of Biosciences, University of Birmingham, Birmingham, United Kingdom

Abstract

The production of recombinant biopharmaceuticals is a billion-dollar industry, with many high-value proteins expressed in the bacterium *Escherichia coli*. Many recombinant protein production (RPP) systems enable the synthesis and purification of large amounts of soluble recombinant protein. However, the expression of difficult protein targets (*e.g.*, membrane proteins) using these RPP systems may be too high for cells to cope and adequately fold protein. This can result in the production of inclusion bodies, target degradation, or even cell death. Many 'tricks of the trade' can be employed to slow down RPP and increase the level of soluble product, *e.g.,* lowering the growth temperature, using a weaker promoter or a lower copy number plasmid. However, this can mean re-cloning the target gene into another expression system, wasting time and resources. To avoid these issues, we have engineered the *E. coli lite* strains, which allows users to take RPP expression plasmids and immediately lower expression levels, quickly facilitating the production of problematic proteins. Using these strains, we examine the expression of targets, such as green fluorescent protein (GFP) and human growth hormone (hGH), and demonstrate that the *E. coli lite* strains are compatible with many commonly used expression systems (*e.g.*, the T7 RNA polymerase-, *lac*- and *araBAD*-based RPP systems). In particular, we show that the *E. coli lite* strains are ideally suited to expressing toxic membrane proteins, enabling membrane targeting of product, without causing cell death.

B131

Pathogens on Plastics: rapid colonisation drives enhanced virulent and persistent phenotypes

Michael Ormsby ORCID iD[1,2], Luke Woodford ORCID iD[1], Rosie Fellows[1], Hannah White[1], Richard Quilliam ORCID iD[1]

[1]University of Stirling, Stirling, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom

Abstract

Environmental plastic pollution provides a substrate for microbial biofilms, creating a "plastisphere" where both pathogenic and commensal bacteria can thrive. This plastisphere can act as a reservoir that facilitates the spread of pathogens throughout ecosystems, potentially increasing human exposure to these microbes. In order to bind to environmental plastic waste, pathogens need to be in close contact with it; therefore, understanding how rapidly pathogens can bind to plastics and the temporal colonisation dynamics of the continual cycling between the plastisphere and the environment are important factors for quantifying the persistence of human pathogens.

Using simulated environmental conditions, our study reveals that pathogenic *E. coli* O157 can rapidly attach to plastics within 30 minutes and persist at infectious concentrations for at least 21 days. More concerningly, repeated cycles of attachment to and detachment from plastic surfaces appear to select for enhanced persistence and adaptation, resulting in *E. coli* variants with increased biofilm formation and tolerance to antibiotics. This repeated colonisation process not only supports long-term survival but also drives the evolution of traits associated with greater virulence and resistance.

These findings suggest that environmental plastics, through continuous colonisation and cycling, may select for hardier pathogenic strains, thus amplifying the health risks linked to plastic pollution. The adaptation of pathogens like *E. coli* O157 on plastics underscores the role of plastic waste as a co-pollutant in natural settings, one that can facilitate the emergence and dissemination of more persistent, resilient pathogens with implications for both environmental and human health.

B132

Engineering algae-bacteria consortia for complete mineralization of toxic textile dyes

Austin Semple ORCID iD, Jagroop Pandhal ORCID iD

University of Sheffield, Sheffield, United Kingdom

Abstract

The widespread use of azo dyes in industry poses significant environmental hazards, as many dyes resist conventional wastewater treatments and can release toxic by-products. Microbial approaches to dye effluent treatment often require sequential anaerobic and aerobic conditions to achieve complete dye degradation, complicating industrial applications. Dyes are also useful model organic pollutants for studying microbial interactions in biodegradation, allowing development of high-throughput screens. This study aims to develop a single-stage, aerobic system combining microalgae and bacteria to achieve the complete degradation of Congo Red, a model azo dye, without requiring sequential anaerobic-aerobic treatment. Using *Chlorella sorokiniana* as the primary algal component, initial work focuses on enriching compatible bacterial strains capable of utilising Congo Red degradation intermediates from environmental samples. The spent growth media from algal cultures, post-Congo red decolourisation, served as an enrichment medium for isolating bacteria which target dye by-products as a primary carbon source. The same spent media was analysed with LC-MS to identify products yielded by algal decolourisation. To infer the ability of isolates to utilise Congo red by-products, growth was compared in the spent media of algae grown in the presence and absence of Congo red. Combinatorial screening of algae and bacterial isolates reveals candidate consortia for scale-up, to optimise degradation efficiency by varying environmental conditions. Future work will focus on characterising interactions of interest with 'omics tools. This project offers a potential solution for real-world wastewater treatment applications by integrating pollutant breakdown within a single, aerobic system, leveraging algae-bacteria mutualism.

B133

Narrow spectrum antibiotics for the prevention and treatment of soft-rot plant disease

Kirstyn Beattie ORCID iD[1], Katherine Baxter ORCID iD[2], Khedidja Mosbahi[1], Elizabeth Cairns[1], Janet Laird[2], Leighton Pritchard ORCID iD[1], Joel Milner ORCID iD[2], Daniel Walker ORCID iD[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Glasgow, Glasgow, United Kingdom

Abstract

Worldwide losses to plant diseases are conservatively estimated at approximately US $150 bn, of which about one third are attributable to bacterial infections. Soft-rot plant disease is caused through the infection of plants by bacteria such as *Pectobacterium* spp. and can occur in a range of economically important crops such as potatoes.

In many cases, good sources of resistance for breeding are not available and the chemicals used to prevent spoilage are increasingly deemed environmentally unacceptable. The use of broad-spectrum antibiotics in the food chain is also undesirable as this can lead to the selection of antibiotic-resistant strains of bacteria that may ultimately cause infections in humans. Bacteriocins are potent naturally produced protein antibiotics that have a narrow-killing spectrum and could be deployed to target a specific pathogen while leaving the wider plant and soil associated microbiomes intact.

Our current work focusses on the identification, production and testing of novel bacteriocins targeting *Pectobacterium* spp. We are using a range of bioinformatic, genomic and biochemical tools to determine the mechanism of action of identified Pectobacterium targeting bacteriocins. We have also tested the efficacy of bacteriocins in soft-rot plant disease models using an image-based potato tissue infection assay.

B134

The Effect of Reduced Nitrite and Alternative Compounds on the Inhibition of *Salmonella Typhimurium* in Cooked Deli-style Ham

Grace Kennedy[1], Artemis Zavitsanou[2], Declan Bolton[2], Paul Whyte[1]

[1]University College Dublin, Dublin, Ireland. [2]Teagasc, Dublin, Ireland

Abstract

Nitrites are food additives used in processed meats for colour, flavour and microbial safety. Nitrites inhibit pathogenic bacteria including *Clostridium Botulinum, Listeria monocytogenes* and *Salmonella Typhimurium*. Concerns regarding nitrites have arisen due to the health risks associated with the endogenous chemical formation of carcinogenic compounds known as N-nitrosamines. In response, the European Union (EU) has reduced the legal limit for nitrite addition to 120 ppm. This study aimed to investigate the effect of the new EU limit of 120 ppm nitrite on the growth of *Salmonella Typhimurium*, a meat-associated pathogen and contributor to foodborne outbreaks. The following treatments were prepared in cooked deli-style ham samples: H1 (Control), no nitrite added; H2, (Commercial) 120 ppm nitrite, 2.5% salt and 1000 ppm ascorbate; H3, 2.5% salt, 1000 ppm ascorbate and no nitrite; H4, 4% salt, 1000 ppm ascorbate and no nitrite. Samples were incubated at 4°C and 20°C (temperature abuse). At 4°C, there was no growth but the bacteria remained at the inoculated level of 2 log/cfu. At 20°C, inhibition was only observed with ham formulation H4. It was concluded that 120 ppm nitrite does not inhibit Salmonella growth, however, 4% salt and 1000 ppm ascorbate would assure food safety.

B135

Assessment and development of antibacterial paint coated steels for indoor use.

Rupika Gulati ORCID iD[1], Christopher Mills[2], Nicole Robb[1], Freya Harrison[1], Stuart, R Coles[1]

[1]University of Warwick, Coventry, United Kingdom. [2]Tata Steel, Swansea, United Kingdom

Abstract

A combination of the COVID-19 pandemic and rise in healthcare-associated infections has resulted in heightened interest in infection control measures. Studies have shown a link between contaminated surfaces and infection transmission, with some bacteria surviving for months.

Antibacterial coatings have the potential to reduce microbial transmission. However, the assessment of antibacterial coatings relies heavily on the standard ISO 22196. Other standards such as ISO 14698 and ASTM G21 which cover biocontamination control and fungi resistance exist but none of these standards assess longevity and suitability of an antibacterial surface. In this project, we are investigating, if enough is done to ensure a final consumer product is robust and fit for purpose. Following this, if a stringent pipeline is more insightful into compound suitability for a coated surface.

We have investigated the use of metals, as antibacterial alternatives, which can be added into coatings. Several methods, including ISO 22196 and variants of this protocol that determine activity over time, to assess the antibacterial and antibiofilm properties of over 30 metal-based compounds, including nickel, silver, copper and cobalt against *P. aeruginosa PAO1* and *S. aureus USA 300.* Initial findings indicate that copper acetate and silver chloride are amongst a select few to show promising results, with MIC results of no higher than 2mg/mL. We therefore selected some compounds for further antibacterial testing, incorporating them into acrylic and polyester based paints. These findings will help ascertain if different assays will produce alternate outcomes when determining longevity and suitability for antibacterial coated surfaces.

B136

Optimizing Whole Genome and Antibiotic Resistance Hybridization Baits for Enhanced Outbreak Surveillance and Response

Jordyn Upton, Marissa Howard, Minhea Mangalea, Tapati Mazumdar, Anthony Harrington, Margaret Williams, Allison Perry-Dow

Centers for Disease Control and Prevention, Atlanta, USA

Abstract

Sensitive and rapid pathogen detection is critical for identifying reservoirs and directing effective infection control interventions, especially as antimicrobial resistance (AR) in healthcare settings poses a growing public health threat. Traditional culture methods, however, are labor-intensive, time-consuming, and often miss low-abundance pathogens. In this study, we evaluate myBaits® hybridization panels from Daicel Arbor Biosciences® to assess their utility in increasing our ability to detect pathogens and their resistance genes from the healthcare environment. The first is a commercially available myBaits® panel for AR gene detection, with contrived environmental samples to simulate hospital surfaces. Next, we designed a series of custom myBaits® panels targeting the core genome multi-locus sequence typing (cgMLST) regions of three pathogens (*Mycobacterium abscessus, Staphylococcus aureus, Klebsiella pneumoniae*), covering 20,000 loci per pathogen, to evaluate their utility in surveillance. Sequenced pathogens with well-characterized AR profiles were included in the samples to evaluate the effectiveness of the myBaits® panels in capturing target genes. Bait-capture experiments focused on minimizing human DNA crossover during hybridization while maximizing novel AR and target cgMLST gene reads. Our findings suggest that increasing the hybridization temperature from 62℃ to 68℃, yet lowering post-capture binding to 65℃, enhances the specificity of gene and pathogen capture, and reduces off-target human DNA contamination. These optimized conditions demonstrate the potential to refine hybridization protocols for more reliable surveillance of healthcare-associated pathogens, including in complex samples such as wastewater, and for targeted capture of critical genes during outbreak investigations in the healthcare built environment.

B137

Selection and quantification of key functional genes of some selected bacterial species in a microcosms study for biodegradation of crude oil.

Mustapha Gani[1], Azizur Rahman Mohammed[2]

[1]Sokoto State University, Sokoto, Nigeria. [2]University of Greenwich, Medway, United Kingdom

Abstract

GC-MS and qPCR have been used to facilitate the profiling of metabolites from a wide range of oil materials leading to the wide coverage of comprehensive central pathways involving primary metabolism and the quantification of functional genes responsible for the biodegradation of crude oil components. Therefore, the present study aimed to explore the ability of *Pseudomonas aeruginosa* and *Pseudomonas lurida* for the biodegradation of crude oil. The results of the GC-MS analysis showed an extensive elimination of hydrocarbons of mostly low and medium-chain hydrocarbons. The qPCR analysis was carried out to determine the activity of the functional genes and showed a substantially higher relative fold expression of 4-hydroxybenzoate monooxygenase gene (*Ben*) of $2.1x10^{14}$ fold after the first week (T1) during the biodegradation study with *P. lurida*. However, low relative gene fold of 60.91 for catechol-2,3-dioxtgenase gene (*cat23)* was observed. In the same vein, the relative fold expression of 2156.87 was detected for alkane monooxygenase (*alkB*) gene from a study with *P. aeruginosa*. This is substantially higher than the expression for *cat23* gene and greatly lower than the *Ben* gene. The overall results of this study could evidently prove the environmental application of these bacterial species – *B. endophyticus P. aeruginosa* and *P. lurida* for the management of crude oil polluted environments. Hence, the overall finding from this study could be utilised as a tool to design an engineered bioremediation process to address the long devastating crude oil pollution across the Niger Delta and beyond.

B138

Assessment of the Interactions of the Anaerobic Soil Microbiome with Crop Protection Products under Laboratory and Field Conditions

Lauren Olley ORCID iD[1], Shannon Flynn[2], Lee Higham[2], Emma France[3], Laurence Hand[3], Angela Sherry[1]

[1]Northumbria University, Newcastle, United Kingdom. [2]Newcastle University, Newcastle, United Kingdom. [3]Syngenta, Bracknell, United Kingdom

Abstract

Crop Protection Products (CPP) e.g. fungicides, herbicides, pesticides are used globally to control pests and disease in agriculture. To register CPP, persistence in soils must be determined to ensure CPP will not pose a risk to human health or the environment. Persistence testing guideline 307, from the Organization for Economic Co-operation and Development (OECD), recommends soils must be tested under both aerobic and anaerobic conditions in a laboratory. However, substantial discrepancies have been observed between laboratory and field studies, with CPP often degrading faster in the field. Discrepancies may result from deviations from natural conditions during OECD testing, including the loss of key anaerobes during soil handling. As a result, laboratory OECD testing may misrepresent the persistence of CPP in the environment.

Preliminary work investigated microbial communities from anaerobic waterlogged soils and compared findings with existing microbial datasets to gain an understanding of the microbial communities present in anaerobic agricultural soils. This dataset forms the baseline against which we will determine changes in the anaerobic soil microbial communities when subjected to different stages of soil handling during OECD 307 testing procedures.

Outcomes aim to provide recommendations for improvements to OECD 307 guidelines for the handling of anaerobic soils during regulatory testing. Furthermore, the interactions of CPP with anaerobic soil microbial communities will be elucidated to resolve differences in persistence between laboratory and field trials. Through the use of standard regulatory studies combined with microbial ‘omics, improvements in conceptual models of the environmental fate of CPP are anticipated.

B139

Microbial analysis in a shelf-life study of *Genypterus capensis*

Michael Graz

Biophys Limited, Mamhilad Park, United Kingdom. University of Copenhagen, Copenhagen, Denmark

Abstract

Thirty percent of global fish production is lost due to post-harvest microbial spoilage by environmental bacteria and contamination by post-harvest activities such as handling, gutting, and filleting. Only a small percentage of the initial microbiota of captured fish cause the spoilage with Hydrogen Sulphide Producing Bacteria (HPB) responsible for off-odours and off-flavours leading to the product loss.

The aim of this study was to determine the rate of spoilage of *Genypterus capensis* during post-harvest, and post processing, storage in flake-ice after capture in the South Atlantic during two seasons. We assayed for HPBs as well as the standard indicators of spoilage and contamination used to determine fish safety and quality

On a log scale, the total aerobic bacterial numbers increased by 4.5 in summer and by 2 in winter over the 20-day study period in unprocessed and filleted fish. The total aerobic counts showed seasonal variation, due to lower initial bacterial load in winter and increased environmental temperatures in summer.

Within the HPBs, *Clostridium perfringens* and *Shewanella putrefaciens* were not detected in any sample during the study. *P.aeruginosa*, however, was found to increase by 3 on a log scale by day 14 of the study in both summer and winter and was the most effective indicator of spoilage during the storage process.

The study showed that standard microbiological testing of fish is less effective in predicting spoilage than non-standard testing for HPBs. Supplemental techniques should be employed to predict spoilage and reduce loss of products.

B140

Investigating microbial community functionality in wetlands for greenhouse gases emission management

Julia Ghantous[1], Raphy Zarecki[2], Gon Carmi[2], Shiri Freilich[2], Keren Yanuka-Golub[3]

[1]Tel-Hai College, Kiryat Shemoneh,, Israel. [2]Newe Ya'ar Research Center, Ramat Yishay, Israel. [3]The Galilee Society Institute of Applied Research, Shefa-Amr, Israel

Abstract

Wetlands are considered the largest natural source in the global methane budget, a powerful greenhouse gas (GHG) that is strongly associated with climate feedbacks. Microbial communities play a central role in organic matter breakdown, resulting in methane production by archaeal methanogens. The primary objective of this research is to investigate how wetland microbial ecology affects GHG emissions, applying genome-scale metabolic models (GSMM) for the processing of genomic information into predictive models and design of microbial community function. Specifically, the study aims to compare GHG emission profiles across different wetland sites and examine the influence of microbial community composition. This work involved monitoring two natural wetland reserves (Ein Afek and Ein Nimfit, Israel) and one constructed wetland (Kibutz Harduf, Israel). Soil and water samples were collected to monitor physiochemical parameters and microbial dynamics through 16S rRNA amplicon sequencing. Results showed that the microbial community composition varied among the sites. While methanogens and nitrogen-fixing bacteria were prevalent in Ein Nimfit, fermenter bacteria dominated in Ein Afek. In contrast, carbohydrate degradation processes were observed in the constructed wetland. This variation corresponded to GHG emission profiles detected in bottle-incubations and long-term microcosm experiments. Ein Nimfit exhibited the highest methane production without spiking organic matter, which was explained by the higher abundance of acetotrophic and hydrogenotrophic methanogens compared to other sites. Nevertheless, we were able to significantly mitigate methane emissions following metabolic additives predicted through GSMM. Thus, investigating various wetland sites and associated microbial functionality provide insights into potential strategies for managing GHG emissions.

B141

High Altitude Himalayan Wetland: A Boon or Bane from a Climate Change Perspective

Om Prakash Sharma ORCID iD[1], Shweta Kadam[2], Prakash Rao ORCID iD[1], Rajesh Joshi[3], Indrajeet Tyagi,[4], Vikas Kuwar[4]

[1]1. Symbiosis Centre for Climate Change and Sustainability, Symbiosis International (Deemed University), Pune, India., Pune, India. [2]1. Symbiosis Centre for Climate Change and Sustainability, Symbiosis International (Deemed University), Pune, India, Pune, India. [3]G.B. Pant National Institute of Himalayan Environment, Sikkim Regional Centre, Gangtok, Sikkim, India., Gangtok,, India. [4]Zoological Survey of India, Kolkata, India, Kolkata, India

Abstract

The Indian Himalayan Region (IHR) constitutes a unique ecosystem. High-Altitude Wetlands (HAWs) of Himalya have special ecological significance, located above 3000m above mean sea level (amsl), fed by snow and glaciers and experience minimum anthropogenic and animal intervention. They are mostly hypoxic due to low oxygen concentration and experience lower temperatures throughout the year. Understanding the biogeochemistry and microbiology of HAW is imperative from climate change perspective. To investigate the biogeochemical and microbiological studies four different HAWs located in Uttarakhand, Arunachal, Sikkim and Ladakh region of India are selected. Water and sediment samples from 3 different lakes are collected and transported to the laboratory. Sediment samples were used to analyze for depth-wise carbon profiling to understand the variations in carbon capture efficiency of different high-altitude Himalayan wetlands. Collected samples were also subjected to enrich and isolate the psychrophilic bacteria, archaea and fungi and to screen for industrially important psychrophilic enzymes, Furthermore, a comparative rate of greenhouse gases (CO_2, CH_4 and N_2O) release will be measured using anaerobic microcosm. Microbiological profiling will be conducted to reveal the community of methanogens and denitrifies. Obtained data indicates that the sediment of these lakes stores immense carbon and unique microbial diversity. Twenty different strains of psychrophilic bacteria and 5-different fungal strains were enriched and isolated. Taxonomic and physiological investigation of isolated culture is under investigation. Obtained data indicate that high-altitude Himalayan wetlands harbor unique microbial diversity and show substantial variations in carbon storage.

B142

Microalgal biorefinery from sugarcane vinasse: growth, biomass recovery and by-products biodigestion

Reinaldo Bastos ORCID iD[1], Sara Rezende[1], Felipe Barboza[2], Mariana da Silva[1]

[1]Federal University of São Carlos (UFSCar), Center of Agricultural Sciences (CCA), Araras, Brazil.
[2]Centro Universitário da Fundação Hermínio Ometto (FHO), Araras, Brazil

Abstract

Microalgae biorefineries have emerged as promising process for utilizing carbon-rich components to produce a variety of bioproducts. *Phormidium autumnale*, a prokaryotic filamentous, has demonstrated notoriety and tolerance to grow in industrial wastewaters while producing microalgal oils, alternative resources for obtaining biodiesel as 3rd generation biofuels. Vinasse is the main wastewater from sugarcane processing, generated at a high temperature with a strong odor, dark brown color, acidic pH, and high levels of organic matter and potassium. In Brazil, fermentation-distillation technologies generate approximately 10 L of vinasse per liter of ethanol, representing approximately 280 billion liters of wastewater in 2024/25 sugarcane harvest. After microalgae growth and cells separation, oil extraction to obtain biodiesel generates a “fat-free biomass”, which can be sent for biodigestion with energy generation, closing an use cycle of all by-products. In this context, the aim of this research was to evaluate the growth of *P. autumnale* in sugarcane vinasse, biomass recovery by centrifugation and biodigestion of the remaining cells and wastewater. The cultures achieved μ_{max} 0.012 h^{-1} and maximum volumetric productivity in biomass 11.9 mg $L^{-1}h^{-1}$ in vinasse with minimum cooling (down to 45^{o}C). Over 90% of the biomass was recovered using low speed centrifugation (13 *xg*) followed by oil extraction. Co-biodigestion of the remaining cells and vinasse demonstrated the production of more than 50% of CH_4 after 10 days. The results indicate the potential of integrating this microalgal biorefinery with sugarcane processing in Brazil.

B143

Monitoring Wastewater Microbial Populations to Gain Better Understanding of Biodegradation Standard Method Outcomes

Kali Panella, Qiong Wang

International Flavors & Fragrances, Wilmington, USA

Abstract

Accurate measurement of the biodegradability of new commercial products is critical for their proper classification and regulation, as well as for moving industries towards more-sustainable solutions. Standardized methods such as those set by the Organisation for Economic Co-operation and Development (OECD) are widely used to measure the biodegradability of such substances.

When following certain standard methods for measuring biodegradability, inoculum dosing may be based on proxy values like total suspended solids in the inoculum source. This can make it challenging to control for certain test variables including inoculum diversity and viable cell concentration. Because changes in these variables may cause test-to-test variations and improper classifications of materials, we are interested in improving characterization of the inoculum source and better understanding how this may impact test results. Inoculum characterization before and after biodegradation of a particular material may also contribute to understanding of the mechanism of biodegradation that has taken place in a given test.

Next-generation sequencing (NGS) can be employed to better understand the impact of the inoculum on standard method test results. This study will discuss the microbial (both bacterial and fungal) population changes in a United States East Coast wastewater source, both before and after biodegradation of microcrystalline cellulose, a material that may be used as a slow-degrading positive control in biodegradation tests.

B144

How does the third replicon of Burkholderia cepacia complex species impact preservative tolerance?

Brooklyn Rowlands[1], Eshwar Mahenthiralingam ORCID iD[1], Stuart Campbell-Lee[2]

[1]Cardiff University, Cardiff, United Kingdom. [2]Unilever, Port Sunlight, United Kingdom

Abstract

Introduction

Burkholderia species were mentioned in 17% of European home and personal care product contamination notifications in 2023, leading to revenue loss and potential harm to human health. The third replicon of *Burkholderia* bacteria has been previously shown to be implicated with virulence and pathogenicity. In this work, we asked if the third replicon had a role in preservative tolerance, as understanding the mechanisms bacteria employ to evade common preservatives is essential to developing successful preservative strategies.

Methods

Third replicon deletion mutants (ΔC3) of three industrial *Burkholderia contaminans* strains and one unclassified *Burkholderia* strain were generated using a previously described method. The minimum inhibitory concentration (MIC) of phenoxyethanol and sodium benzoate for parental and ΔC3 mutants were compared. Longer term-incubation experiments in minimal media with sub-MIC levels of preservative were used to determine if the third replicon conferred fitness against common preservatives for *Burkholdeira lata* strain 383 over 28 days.

Results

Successful mutants of *B. contaminans* strains BCC0254, BCC1315, BCC1323 and unclassified species BCC1314 were created. The MIC of phenoxyethanol and sodium benzoate for these ΔC3 mutants was not affected compared to parent strains. However, on longer incubation with lower levels of these preservatives, the B. lata ΔC3 mutant had reduced survival on exposure to sodium benzoate after 7 days.

Conclusion

ΔC3 *Burkholderia* mutants did not show alterations in preservative tolerance when exposed to commonly used preservatives. Further work is being carried out to determine if this replicon may confer preservative tolerance and survival over a longer growth period.

B145

Soil survival and re-emergence: the continued threat of plague.

Luke Shipley ORCID iD, Elizabeth Ison, Steve Atkinson

University of Nottingham, Nottingham, United Kingdom

Abstract

Yersinia pestis is a Gram-negative bacterial pathogen and the causative agent of plague, a deadly infection usually found in small mammals and hematophagous insects, primarily fleas. Plague epidemiology is characterized by sporadic outbreaks of disease, typically followed by quiescent periods of 2–5 years; however, instances of prolonged dormancy have also been reported, with plague reemerging in foci decades after the last known case. The exact reservoir for *Y. pestis* during quiescent periods has not been conclusively determined. It has been hypothesized that *Y. pestis* is capable of a 'sit-and-wait' lifestyle in soils, a trait inherited from its most recent ancestor, *Yersinia pseudotuberculosis*, an enteric human pathogen known to persist in soils. Free-living-amoeba are ubiquitous in soils and have been shown to act as cellular reservoirs for several human pathogens, including *Legionella pneumophila* and *Vibrio cholerae*. Previous studies indicate*Y. pestis* can survive intracellularly within amoeba for 2–5 days, with intracellular replication of the bacterium reported in *Dictyostelium discoideum*, raising the prospect of an amoeboid reservoir for *Y. pestis* in soils.

Our initial investigations indicate *Y. pseudotuberculosis* is capable of surviving in coculture with *Acanthamoeba polyphaga* for a maximum testing period of one week. Advanced microscopic imaging of these cocultures also revealed that, if phagocytosed, *Y. pseudotuberculosis* can persist intracellularly within the amoeba for up to 48 hours. Future work will aim to characterize the ability of *Y. pestis* to resist amoebic predation and lysis, and to develop novel soil survival models incorporating both *Y. pestis* and *A. polyphaga*.

B146

A novel method for monitoring biofilm build-up in industrial laundry equipment

Jake Dunlop ORCID iD, Raymond Allen, Katie Laird

De Montfort University, Leicester, United Kingdom

Abstract

Biofilm formation is a potential issue in healthcare industrial laundries. Microorganisms present in biofilms can potentially induce degradation of surfaces to which they are attached, impacting upon the tunnel washer's performance and stability. Identifying the presence of biofilms within equipment is therefore paramount, however, no methods are currently available to monitor biofilm formation in difficult to access areas of industrial tunnel washers.

Aim: To develop a new approach to detect and quantify biofilm formation within healthcare industrial laundry machines.

Methods: Specially engineered frames were created for the suspension of stainless-steel coupons within two different areas within an industrial laundry machine; a tunnel washer module (used for disinfection of laundry) and a recycled wastewater tank. After one month of exposure, coupons were assessed by bacterial enumeration, scanning electron microscopy (SEM) to detect the presence of biofilms, and 16S metagenomic analysis.

Results: Frames were successfully engineered for detection of biofilms within tunnel washers. Biofilm formation was detected in the wastewater tanks but not in the module exposed to high temperature (71°C) and chemical disinfection processes. Lint appears to play a role in biofilm formation within the wastewater areas of the laundry machines, with all SEM images from this area evidencing biofilms in association with lint.

Conclusion: Healthcare laundry equipment with difficult to access areas that are used on a continuous basis requires specific engineering solutions for the detection of biofilms. This new approach presents an effective method to monitor biofilm formation and therefore improve industrial laundry machine design and disinfection processes.

B147

Tracking Cefotaxime-Resistant Escherichia coli and Vancomycin-Resistant Enterococci hotspots in surface waters in Newcastle, Northern Ireland.

Calum Cheung ORCID iD[1,2], Patrick Naughton[1], James Dooley[1], Nicolae Corcionivoschi[2], James Brown[2], Conan Bradley[2], Rachel McGroarty[2], Stewart Nairn[2], Cathy Brooks[2]

[1]Ulster University, Coleraine, United Kingdom. [2]Agri-Food and Biosciences Institute (AFBI), Stormont, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a global health concern. Surveillance of AMR bacteria has been well established in healthcare settings in Northern Ireland. However, investigations into the presence of AMR bacteria at designated bathing water sites is limited. Current legislation for monitoring water quality in bathing water does not require investigation of AMR bacteria. This is concerning as water is a medium transporting resistant bacteria and AMR genes and facilitates survival and proliferation of these organisms.

Newcastle, Co Down is a popular seaside town in Northern Ireland with a beach that is a designated bathing water site and, is frequently visited by people from throughout Northern Ireland and beyond. The beach receives the discharge from two rivers that flow through agricultural and urban areas.

In this study, we collected water samples from sites throughout the catchment from September to November 2024. Samples were filtered through cellulose nitrate filter membranes (0.45µm pore size) using an adapted method from ISO-7899-2 for the enumeration of intestinal enterococci and The Standing Committee of Analysts Method for enumeration of *Escherichia coli*. The membranes were placed on tryptone bile X-glucuronide (TBX) agar, TBX supplemented with cefotaxime (4µg/mL), Chromocult enterococci agar and Chromocult enterococci agar supplemented with vancomycin (8µg/mL). The plates were incubated for 24 hours and colonies were counted to calculate the CFU/100ml.

The study found a positive correlation between *E. coli* concentration and CTX-resistant *E. coli*, as well as enterococci concentration and vancomycin-resistant enterococci. Additionally, resistant enterococci was most frequently detected upstream of the urban area.

B148

Antimicrobial activity of essential oil nanoemulsions against foodborne pathogens *Staphylococcus aureus* and *Listeria monocytogenes*

Pierric Louis Désiré Chuppa, Natalia Rodríguez Cánovas, Alfredo Palop, Alberto Garre, Paula M. Periago Bayonas, Enriqueta Garcia-Gutierrez ORCID iD

Polytechnic University of Cartagena, Cartagena, Spain

Abstract

Staphylococcus aureus and *Listeria monocytogenes* are significant foodborne pathogens that pose serious risks of gastrointestinal infections. Effective strategies to reduce and eliminate these microorganisms in food products are essential to enhance food safety. Natural alternatives like essential oils from plant by-products have emerged as promising antimicrobial agents, offering safer and more sustainable solutions than chemical preservatives. Notably, essential oils from rosemary (*Salvia rosmarinus*) and oregano (*Origanum vulgare*) have demonstrated efficacy against various foodborne pathogens, though their specific effects on *S. aureus* and *L. monocytogenes* require further exploration. This study developed nanoemulsions of rosemary and oregano essential oils at different concentrations to evaluate their impact on *S. aureus* and *L. monocytogenes*. Bacterial growth was monitored over 48 hours using a Bioscreen C system, and the data were analysed with R packages. The results showed concentration-dependent bactericidal and bacteriostatic effects on both pathogens. Higher concentrations of the essential oils inhibited bacterial growth entirely, while lower concentrations slowed growth rates. These findings suggest that rosemary and oregano essential oils could be tailored for various food applications, such as reducing contamination during processing or inhibiting microbial growth during storage. This study highlights the potential of essential oils as natural preservatives, providing valuable insights into their role in pathogen control and food safety.

B149

Treasure hunt: Exploring in-situ microbial communities via enhanced cultivation-based approaches

Priyanka More[1], André Antunes[2]

[1]Biology Department, Edge Hill University, Ormskirk, United Kingdom. [2]Institute of Science and Environment, University of Saint Joseph Macao, Macau, China

Abstract

Microorganisms are omnipresent and are active partakers of many bio-chemical processes. Researchers studying these organisms are extensively exploring this treasure for their potential roles in medicinal, industrial, ecological fields etc. Modern microbiology uses both molecular and culture-dependent techniques, primarily the former. However, traditional microbiological methods of cultivation are known to recover less than 1% of the total microbial species, as the majority of these cultures may have growth strategies which are not observed when traditional cultivation indicators are used. Microbial growth is easily missed by conventional cultivation methods because of inadequate knowledge of their growth requirements which could be selective at confined concentrations and long incubation periods. Many microbial strains are still uncultured and the biochemical and physiological characteristics of many of these microbes are still unknown. It is also beneficial to have this missed population of valuable microbial isolates in our culture collection, given the range of applications for industrial, medical sciences and laboratory healthcare use.

Growing uncultured microbes has been one of the big challenges to the microbiology research community, and hence needs to be discussed and revisited more often. In our study attempts were made to partially address the current gap in cultivation techniques by developing a Gel-stabilised gradient platting technique that artificially recreates naturally occurring interfaces such as those with diverse salinity and pH ranges. Here we present our recent results of developing and applying this modified platting technique together with an overview and discussion of microbe cultivation challenges and the potential of enhanced cultivation strategies

B150

Use of remaining sugarcane vinasse from *Phormidium autmnale* growth for producing chitosan-based particles

Mariana Altenhofen da Silva, Gabriel Rocha Kaiser, Reinaldo Gaspar Bastos

UFSCAR, Araras, Brazil

Abstract

Sugarcane vinasse is the most expressive wastewater from ethanol production, and the fertigation of nearby sugarcane crops configures its main destination. However, indiscriminate application of vinasse can lead to environmental impacts, prompting the need for alternative uses and treatment methods. One promising approach is utilizing vinasse as a growth medium for microorganisms, owing to their fast-growing ability and the possibility to accumulate biomolecules of economic and industrial interest. The filamentous microalgae *Phormidium autumnale* stood out for its viability to grow in agro-industrial effluents suggesting a possible integration into a biorefinery system. After microalgal growth and biomass separation, the remaining vinasse still contains significant nutrient levels that could be repurposed to produce biodegradable particles intended as agricultural fertilizers. This approach would enhance the sustainability of the production chain by obtaining a novel bioproduct and expanding the applications of vinasse. Bio-based polymers, such as chitosan, have been used for developing systems that can delay or control the release of fertilizers and other agrochemicals ensuring more efficient applications. This study aimed to produce and characterize chitosan-based particles enriched with the remaining vinasse from *P. autumnale* growth. Spherical chitosan beads were produced by ionotropic gelation, using tripolyphosphate 5% as a crosslinking agent, and drying at 30°C/24h. Vinasse increased the particle size and enhanced their compression strength, possibly due to the higher concentration of soluble solids and residual biomass from the wastewater. These particles could be used as fertilizers in several crops, allowing nutrient recycling, and offering a more adequate destination for this wastewater.

B151

Use of Custom Nanotrap® Particles to improve recovery of healthcare-associated pathogens from the built environment

Olivia Yancey[1], William Norfolk[2], Monica Chan-Riley[1], Marissa Howard[1], Paige Gable[1], Kristen Clancy[1], Margaret Williams[1], Kristen-Allison Perry-Dow[1]

[1]Centers for Disease Control and Prevention, Atlanta, USA. [2]University of Georgia Athens, Athens, USA

Abstract

Detecting bacterial pathogens in the healthcare environment from low bioburden samples can be challenging. Custom Nanotrap® Particles (CNPs) (Ceres Nanosciences, Manassas, VA) are a cost-effective method to efficiently concentrate and recover pathogens. CNPs can concentrate a wide range of sample types, including primary environmental samples and liquid media, to capture whole-cell pathogens of interest. Using qPCR, we evaluated the efficacy of CNP capture versus traditional culture-dependent methods on complex contrived samples and healthcare outbreak samples.

Known quantities of *Mycobacterium abscessus* (MA) or *Klebsiella pneumoniae* (KP) were independently added to contrived samples with and without a simulated environmental contamination matrix (ECM) and were treated with CNPs to determine pathogen recovery.

CNPs detected significantly ($p < 0.05$) more MA (23.59% and 16.04%) than traditional culture-dependent methods (6.55% and 7.94%) for 30 mL and 50 mL samples, respectively. CNPs increased recovery rates of target pathogens from contrived and outbreak response samples compared to traditional workflows. In a KP outbreak using broth enrichment, only 2% of 59 broths were confirmed positive (1/59). Treatment with CNPs confirmed 7% of 59 (4/59) samples were positive.

The addition of ECM reduced the recoverability of both MA and KP (1-3 log-change in quantity) in controlled laboratory experiments. This reduction was expected following the addition of inhibitory compounds within the ECM, designed to mimic the contamination levels of true environmental samples in a contrived laboratory. CNPs are a cost-effective method using simple magnetic separation to improve pathogen recovery during outbreak investigations.

B152

Unraveling genome plasticity and acquisition of hyaluronic acid capsule trait in an environmental GRAS isolate -Bacillus subtilis K3C- through whole genome de novo sequence analysis.

Simran Gagneja, Neena Capalash, Prince Sharma

Panjab University, Chandigarh, India

Abstract

Genome plasticity is central to adaptation under selective pressure conditions and has attracted a considerable interest over the years. The expression of capsule trait is often associated with virulence and extended environmental breadth. Virulent *Streptococcus* spp. are known to express hyaluronic acid as capsular material that has commercial utility. In our study, we have identified an environmental GRAS strain producing hyaluronic acid as capsular material and evidences suggesting the gene cluster acquisition and expression were collected using whole genome sequence analysis. Our findings suggest that the strain K3C has acquired different phenotypic traits through transformation-mediated HGT process. Intact prophage regions were identified in the genome, however, *has* gene cluster was not integrated into the prophage regions, rather genes were integrated in different chromosomal regions. The reticulogram inferences revealed that *has* gene cluster acquired by the strain was phylogenetically closer to the *has* operon present in *Streptococcus equi* and *S. pyogenes.* The phyloproteome analysis of hyaluronan synthase gene suggests that it has a common firmicutes ancestry. The prevalence of *has* operon has been reported across different *Bacillus* species. *has* operon was acquired by *B. anthracis, B. cereus* and *B. tropicus* through plasmid-mediated HGT. Overall, our findings shed light on genome plasticity observed in strain evolution. The surface morphology analysis and structural characterization of purified capsular material also validated our data.

B153

Plant growth-promoting bacteria inhibit fungal plant pathogens

Josephine Giard ORCID iD[1], Angela Feechan ORCID iD[2], Stephen Euston ORCID iD[1,3], Michele Partipilo ORCID iD[4], Pablo Iván Nikel ORCID iD[4], Leena Kerr ORCID iD[2], Ross Alexander ORCID iD[2]

[1]Institute of Biological Chemistry, Biophysics and Bioengineering, School of Engineering and Physical Sciences, Edinburgh, United Kingdom. [2]Institute of Life and Earth Sciences, School of Energy, Geoscience, Infrastructure and Society, Heriot-Watt University, Edinburgh, United Kingdom. [3]Dept. of Physics, Toronto Metropolitan University, Toronto, Canada. [4]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kongens Lyngby, Denmark

Abstract

Climate change brings severe agricultural challenges as crops are exposed to increased levels of both abiotic (drought, waterlogging) and biotic stresses (fungal diseases). A large body of literature now shows that by enriching soils with bacteria possessing plant growth-promoting (PGP) characteristics, plant resilience against pathogens can be improved. However, to identify beneficial bacteria, it is important to first investigate their PGP potential. Here, we assessed the PGP potential of selected (novel) soil bacteria of local (UK) and global origin, including *Pseudomonas*, *Mucilaginibacter*, and *Paenibacillus* species. Employed approaches included assessment of the production of PGP metabolites, solubilisation of (in)organic compounds, enzyme activity, tolerance of stressors, biofilm formation, and nitrogen fixation. We found that all investigated bacteria possess some but not all of the PGP properties. *Ps. protegens* DTU 9.1, *Ps. koreensis* C63, and *Ps. koreensis* D70 showed the greatest PGP potential while *M. gossypiicola* and *M. frigoritolerans* were the least active. Subsequently, we assessed the potential of these bacteria to inhibit the growth of several different fungal pathogens (*Botrytis cinerea*, *Ramularia collo-cygni*, and *Zymoseptoria tritici*). We found that a majority of bacteria were able to inhibit the growth of at least one of the fungal pathogens. Analysis of genomic sequencing data suggests that some of these bacteria produce novel antifungal compounds. We are currently working to purify and identify these novel antifungal compounds, and to test the bacteria's potential to be used in biocontrol strategies to prevent and treat plant diseases.

B154

Sustainable disinfection - using a PAN catalyst to improve disinfectant efficacy against norovirus

Brandon Moulds, Katherine Huddersman, Maitreyi Shivkumar

De Montfort University, Leicester, United Kingdom

Abstract

Norovirus is a highly transmissible non-enveloped virus, and a major pathogen contributing to gastroenteritis worldwide. The use of disinfectants for infection prevention is critical to reduce transmission, especially in hospitals and low-income settings. Here, we investigated the reusability and antiviral effects of an iron-impregnated polyacrylonitrile (PAN) catalyst system on disinfection of a model murine norovirus (MNV-1) using hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl). We also assessed the development of resistance in MNV-1 upon repeated treatment with H_2O_2, and the ability of the PAN catalyst system to overcome this.

Catalytic activity was determined by degradation of RO-16 orange dye using UV-VIS spectrophotometry. Reusability was investigated by repeated RO-16 degradation assays and inspecting catalyst structure using AAS and SEM-EDX. Antiviral efficacy of H_2O_2 and HOCl in the presence of the catalyst against MNV-1 was determined using the BS EN 14476:2013+A2:2019 quantitative suspension test methodology, with infectious viral titre quantified for a range of concentrations and treatment times. To assess development of resistance, MNV-1 was serially exposed to H_2O_2 for 10 cycles, and susceptibility to disinfection tested at passages 0, 5 and 10.

H_2O_2 activity was significantly improved in the presence of the PAN catalyst by increasing degradation of RO-16 dye. The PAN catalyst also increased disinfection efficacy, with significantly lower concentrations of H_2O_2 and HOCl required for inactivation of MNV-1. Low rates of iron leaching and sustained activity across multiple applications demonstrates the sustainability of the PAN catalyst system. Assessing changes in MNV-1 sensitivity to H_2O_2 is currently on going.

B155

Expression of plastic-degrading enzyme candidates selected by a structure-based bioinformatic screen.

Eloise Gray, Eva Sonnenschein, Matthew Tarnowski

Swansea University, Swansea, United Kingdom

Abstract

Polyethylene terephthalate (PET) is a polyester used for plastic bottle production, which accounts for approximately 8% of global plastic production. Recently, many PETase enzymes have been identified through computational searches based on sequence-similarity. At the same time rapid structure-based similarity searches combined with protein structure prediction have enabled in silico screening of metagenomic data for enzymes which are structural homologs of plastic-degrading enzymes. In this study, we aim to identify plastic-degrading enzymes in novel sequence space using structure-based bioinformatic screening followed by heterologous protein expression and testing. Twenty-three enzymes with significant evidence of plastic degradation were selected as query structures. ESM atlas was used to identify structurally similar enzyme candidates and these were filtered for high structural similarity and low sequence similarity to the query, ready for gene synthesis, expression, purification and testing. ESM returned 22,183 hits from a search of 772 million predicted metagenomic protein structures. These hits were computationally filtered to ten genes encoding enzyme candidates with similar structure but dissimilar sequences to known plastic-degrading enzymes. Structural homologs of seven genes encoding polyester-degrading enzymes and two genes encoding polyolefin-degrading enzymes were synthesised in expression plasmids and transformed into a bacterial strain for testing, which is ongoing. The bioinformatic screening pipeline successfully identified structural homologs of plastic-degrading enzymes in novel sequence space. Our future work will involve expressing and testing these enzyme candidates for plastic-degradation activity and applying them for wastewater bioremediation within a novel wastewater-treatment technology developed by the BMRex consortium (www.bmrex-project.eu/) to minimise microplastic pollution.

B156

Integrating Pathogen Data to Inform Rodent Control Strategies for One Health: Findings from the RODENTGATE Project

Hayley Thompson ORCID iD[1], Steve Belmain[1], Judy Bettridge[1,2], Sophie Bouvaine[1], Daniel Bray[1], Bram Vanden Broecke[3], Florian Huels[4], Jens Jacob[4], Inge Krijer[5], Herwig Leirs[3], Hope Okon[1], Goncalo Silva[1], Vincent Sluydts[3], Anna Szczotka-Bochniarz[6], Marina Voinson[3]

[1]Natural Resources Institute, University of Greenwich, Chatham, United Kingdom. [2]Bristol Veterinary School, University of Bristol, Langford, United Kingdom. [3]University of Antwerp, Antwerp, Belgium. [4]Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut, Braunschweig, Germany. [5]Dutch Pest & Wildlife Expertise Centre, Wageningen, Netherlands. [6]National Veterinary Institute, Pulawy, Poland

Abstract

Rodents are reservoirs for zoonotic pathogens, but less is known about their role in economically important livestock diseases. Anticoagulant rodenticides have been the primary tool for managing rodent pests, but are becoming increasingly restricted due to environmental concerns, risking a potential rise in rodent-borne diseases. The RODENTGATE project integrated pathogen screening with ecological and rodent movement data to inform control strategies.

To evaluate health risks to pigs and poultry, 654 rodents were trapped on farms across the UK, Belgium, Netherlands, Germany, and Poland over two years. Using PCR and qPCR, the prevalence of economically important pathogens, including *Lawsonia intracellularis*, *Brachyspira hyodysenteriae*, *Leptospira* spp., *Salmonella* spp., chicken anaemia virus, and encephalomyocarditis virus, was assessed in rodent tissue samples. Pathogen prevalence varied across countries, farm types, and pathogens, ranging from 0 to 54.3%. Metagenomic sequencing of pig, rodent and environmental samples on a UK pig-arable farm provided additional insights into compartmental differences in bacterial communities and revealed a broader spectrum of pathogens including foodborne and zoonotic threats.

Bluetooth loggers monitored rodent movements and interactions, which, along with disease transmission models, assessed pathogen spread risks under different control strategies. Results indicated there are no differences between environmental and directly transmitted pathogens. It showed that farm cleaning is the preferred control strategy when the host immune response to a pathogen results in long-lasting resistance. In contrast, rodent culling is more effective when the pathogen does not elicit a durable sterilising immunity. These insights can help guide more targeted agricultural rodent control strategies in future.

B157

The effect of salinity on *E. coli* survival when embedded in different wastewater communities

Nina Baskerville, Lihong Zhang, Anne Leonard, Will Gaze

University of Exeter, Penryn, United Kingdom

Abstract

Aquatic environments are important in the transmission of human pathogens. Climate change is predicted to increase heavy rainfall events which can trigger untreated sewage discharges into aquatic environments. This would result in environments polluted with sewage, human-associated pathogens, and other chemical micropollutants. Understanding how long these pathogens can persist in polluted environments is important to estimate human exposure risk. Although salinity is considered a key factor in *E. coli* decay, its importance relative to biotic factors like predation when embedded in treated and untreated wastewater communities is unknown. This research aimed to determine the effect of environmental variables, such as salinity and predation, on human-associated bacteria across aquatic environments.

Wastewater influent and effluent microbial communities were exposed to a range of salinities (0ppt to 33ppt) in both artificial and natural seawater to determine how salinity and predation affected survival. Survival was determined by intermittent isolation of *E. coli* over three months. *E. coli* from influent had significantly higher decay rates as a function of salinity compared to effluent, suggesting that sewage treatment may select for marine-adapted *E. coli* strains. The decay of *E. coli* from both communities was significantly higher in natural conditions compared to artificial. This suggests predation is a key driver of *E. coli* decay in aquatic environments. Isolates will be sequenced to determine how adaptations to different aquatic environments may relate to antibiotic resistance and virulence genes. This research provides insights into pathogen dynamics in aquatic environments and the potential impact of climate change.

B158

A novel 3D-printed stamping device to simulate gloved-hand contact transfer of bacteria to antibacterial copper surfaces

Alexander Cunliffe ORCID iD[1], Emma Chareyre ORCID iD[1], Peter Askew[2], Gillian Iredale[2], James Redfern[1]

[1]Manchester Metropolitan University, Manchester, United Kingdom. [2]Industrial Microbiological Services Limited, Reading, United Kingdom

Abstract

Antibacterial materials are of increasing importance to tackle antimicrobial resistance to traditional forms of microbial control. Current methods of validating candidate materials (e.g. ISO 22196) specify environmental conditions that the material will likely not experience when in use (e.g. relative humidity above 90%), allowing materials that require moisture (e.g. silver nanoparticles) to present artificially prolonged efficacy. A custom-designed 3D-printable stamping device was developed to accurately simulate gloved-hand contact under realistic environmental conditions. The stamping device was designed and printed to be UV sterilisable, then secure nitrile glove sections from a 3D designed and printed table, followed by stamping an inoculated agar surface to acquire a uniform bacterial load (*Staphylococcus aureus*). The device was then stamped (1 kg weight to simulate human touch pressure) on to recipient surfaces (stainless steel and copper) and the survival over time in varying relative humidity conditions measured via total viable count assays. Consistent initial bacterial transfer occurred on each material type with slight but statistically significant differences between materials (lower recovery from copper than stainless steel) in some cases. No significant reduction in bacterial viability was observed on stainless steel coupons, but varying antibacterial efficacy of copper surfaces was observed (lower efficacy at lower relative humidity environments). The variation in bacterial viability highlights not only the importance of moisture to the antibacterial efficacy of some materials, but also the requirement for standardised testing methods (e.g. ISO standards) to incorporate conditions more analogous to those the material would experience when in use.

B159

Rediscovering the Yellow Pigment Synthesized by *Xanthomonas sp.*

Garima Singh ORCID iD, Deepa P. R., Prabhat Nath Jha ORCID iD

Birla Institute of Technology and Science, Pilani, India

Abstract

The *Xanthomonas* group of bacteria are known to exclusively synthesize a unique group of brightly yellow-colored pigment(s) called “xanthomonadin”. Research on this pigment dates back to the early 1960s, identifying it as a halogenated aryl polyene that has potential antioxidant activity due lipid peroxidation-inhibiting properties. However, its practical applications remain unexplored, largely due to incomplete purification and characterization. To address this, we developed a method to extract and purify the yellow-colored pigment from *Xanthomonas oryzae* pv. *oryzae* (XOO) and characterized it using chromatographic and spectroscopic techniques. XOO was cultivated in a bioreactor, and the yellow pigment was extracted from the bacterial pellet using solvent extraction, followed by saponification and multiple wash steps before HPLC analysis. The pigment exhibited a characteristic absorbance at 440 nm and two shoulder peaks. HPLC revealed two major peaks at retention times of 2.5 and 15.7 minutes, both absorbing at 440 nm and LC-MS analysis detected masses 531.85 m/z and 564.89 m/z. In the C1s XPS spectra, a binding energy of 284.2 eV indicated a highly ordered carbon structure, suggesting an extended conjugated system. Additionally, bromine and chlorine were confirmed to be absent. FTIR, ^{1}H-NMR and ^{13}C-NMR data suggested the presence of carbonyl groups, alkenes, and hydrocarbon chains. Overall, our findings suggest that the purified yellow pigment is a non-halogenated compound without an aryl group, bearing close resemblance to carotenoids. This optimized purification protocol enables further exploration of the pigment’s potential applications as an antioxidant and other biological applications.

B160

Marine fungi and seaweed, a match made in industry? Understanding the biology and biotechnological potential of marine fungi bio-processing of seaweeds.

Beth Tindall-Jones ORCID iD[1,2], Luka Wright ORCID iD[3], Sophie Harley[4], Glen Wheeler[1], Mike Allen ORCID iD[2], Steve Michell ORCID iD[2], Michael Cunliffe ORCID iD[1,4]

[1]The Marine Biological Association, Plymouth, United Kingdom. [2]University of Exeter, Exeter, United Kingdom. [3]University of Western Australia, Perth, Australia. [4]University of Plymouth, Plymouth, United Kingdom

Abstract

Marine fungi are commonly found associated with macroalgae, yet little is understood about the biology and potential biotechnological applications of their interaction. Using the marine fungus *Paradendryphiella salina* and brown macroalga *Fucus serratus,* we investigated fungal response to macroalgae state ('fresh' vs. 'desiccated'). *Fucus* discs were inoculated with fungal spores and incubated across a range of temperatures based on average seasonal temperatures in Plymouth. Mycelium cover on the *Fucus* discs and Hyphal Growth Unit (HGU) were used as proxies for fungal growth and morphology. Fungal ß-glucosidase activity was also measured to consolidate the link between HGU and enzyme activity. Results show that mycelium cover was higher on desiccated seaweed than on fresh seaweed as well as positively related to increased temperature. During the first 2 days of growth, HGU was higher on fresh seaweeds. The combined impact of lower mycelium cover and higher hyphal growth unit implies that *P. salina* is unable to digest fresh *F. serratus* material effectively and therefore showing a possible starvation growth response. This growth morphology is also seen in separate carbon starvation control experiments. Results from the ß-glucosidase activity were inconclusive, thus further experimentation using seaweed derived carbon sources and carbon starved controls will be used to validate the changes of HGU on fresh vs desiccated seaweeds. Overall, this study shows that both desiccation and temperature influence fungal growth of seaweed, and further research is needed to explore the how these parameters will impact both this system in its natural environmental and in biotechnological applications.

B161

Application of viromics to assess fecal contamination in the inflow rivers of Taihu Lake, China

Shuang Liu[1,2], Evelien Adriaenssens[3], Revathy Krishanmurthi[3], Alan McCarthy[2], Sekar Raju ORCID iD[1,2]

[1]Xián Jiaotong-Liverpool University, Suzhou, China. [2]University of Liverpool, Liverpool, United Kingdom. [3]Quadram Institute Bioscience, Norwich, United Kingdom

Abstract

Taihu Lake, the third-largest freshwater lake in China, has been under the influence of water deterioration for decades, with faecal contamination being one of the major sources. Different methods including molecular approaches were previously used to assess the fecal contaminations in the Taihu lake and inflow rivers. A previous research has reported that the presence of human faecal-specific crAssphage microbial source tracking markers (MST) in the surface water of the inflow rivers of Taihu Lake. This brought interest to study the viromes in the inflow rivers of Taihu Lake and their distribution in the faeces of animals and humans (sewage). Viral metagenomics, or viromics, is an effective tool to assess the viral community composition without the need for culturing the viruses. In this study, virome analysis was performed on water samples collected from ten inflow rivers of Taihu Lake, faecal samples from various animal hosts, and domestic sewage samples collected around the watershed. The results revealed that the viral communities varied significantly in response to seasons, locations, and hosts. The predominant presence of *Caudoviricetes* in both water and faecal samples reflected their adaptations and to exploit abundant bacterial hosts. Environmental samples displayed a richer diversity of functional pathways than faecal samples, suggesting their more complex interactions in aquatic environments. Additionally, the study uncovered a highly diverse and abundant collection of *Crassvirales* in environmental water and animal faeces, highlighting the potential for the new *Crassvirales* strains to serve as markers for environmental and animal-specific faecal contaminations in aquatic environments.

B162

Engineering *Streptomyces* bacteria for the sustainable manufacture of antibiotics

Andreas Lawaetz ORCID iD[1], Alexa Gannon ORCID iD[1], John Munnoch ORCID iD[1], Daniel Larcombe ORCID iD[1], Claudio Avignone Rossa ORCID iD[2], Paul Hoskisson ORCID iD[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University of Surrey, Guildford, United Kingdom

Abstract

Like many countries, the United Kingdom has begun addressing climate change by seeking to increase sustainable manufacturing. Production of pharmaceuticals currently represents 5% of the UK's carbon footprint with a large contribution coming from the feedstock used in microbial biofactories. Many antimicrobial drugs are the product of complex biosynthetic pathways found naturally in *Streptomyces* bacteria and are manufactured industrially via large scale fermentations. Organic waste streams – such as bread waste – can potentially be used as alternative feedstocks for more sustainable manufacturing, but secondary metabolism is tightly regulated in *Streptomyces* and alternative carbon sources can have a negative impact on antibiotic yield. Clavulanic acid, designated an essential medicine by the WHO, is a potent beta-lactamase inhibitor produced by *Streptomyces clavuligerus.* High titre production strains have been artificially evolved for >35 years by random mutagenesis using chemical and ionising agents. In this work we determine what mutations have driven strains to adapt to specific media and fermentation conditions. With this information we aim to design, build, and test appropriate engineering strategies for the development and construction of high titre *Streptomyces* strains that utilise greener carbon sources, such as bread waste.

B163

An investigation on the suitability of selective isolation techniques for the isolation of Actinomycetes.

Md Anowar Hossain[1], Paul A Lawson[2], Alistair K Brown[3], Amanda L Jones[1]

[1]Northumbria university, Newcastle, United Kingdom. [2]University of Oklahoma, Norman, USA. [3]Newcastle university, Newcastle, United Kingdom

Abstract

Multi-drug resistance (MDR) is a growing global health concern characterised by microorganisms' ability to resist the effects of multiple antimicrobial agents. This phenomenon complicates the treatment of infectious diseases and limits the efficacy of standard therapies. The overuse and misuse of antibiotics in healthcare and agriculture further accelerate the spread of MDR pathogens. Addressing MDR requires a multifaceted approach, including the search for novel bioactive compounds and alternative treatment strategies. The Great Salt Plains is a unique hypersaline environment that presents challenges for the isolation of microorganisms, including actinomycetes, a diverse group of filamentous, Gram-positive bacteria renowned for their prolific production of antimicrobial agents, stand as a cornerstone in this pursuit. Their diverse metabolites exhibit multifaceted bioactivities, including potent antituberculosis, anticancer, immunomodulatory, immuno-protective, antidiabetic, etc. This study investigates the suitability of selective isolation techniques for recovering actinomycetes from the Great Salt Plains. Different selective isolation approaches including saline-tolerant media, addition of gypsum and chemical inhibitors (e.g., antifungals and antibiotics), were tested to suppress competing microbial growth while enhancing the recovery of actinomycetes. Additionally, physical treatments, such as heat pre-treatment, were employed to eliminate non-target microorganisms. Results indicate that using high-salinity media combined with gypsum, selective inhibitors, and pre-treatments significantly improved the yield and purity of actinomycetes colonies from hypersaline samples. These findings suggest that tailored selective isolation methodologies are essential for the successful recovery of Actinomycetes from extreme environments, which hold potential for the discovery of novel bioactive compounds and industrially relevant enzymes.

B164

Data Driven Discovery of Lasso Peptides with Therapeutic Applications

Josephine Giard ORCID iD[1,2], Ross Alexander ORCID iD[1], David Smith ORCID iD[2], Leena Kerr ORCID iD[1]

[1]Institute of Life and Earth Sciences, School of Energy, Geoscience, Infrastructure and Society, Heriot-Watt University, Edinburgh, United Kingdom. [2]Institute of Biological Chemistry, Biophysics and Bioengineering, School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh, United Kingdom

Abstract

Antimicrobial resistance is one of the major global health threats and to combat it, there is a need to keep discovering novel antimicrobials. A promising target for novel drug discovery are lasso peptides, a class of ribosomally synthesised and post-translationally modified peptides (RiPPs). Their unique topology provides them with high stability against proteases and heat degradation, and they are known to possess a wide range of functions including antimicrobial, antiviral, and anticancer activity. Here, we aim to discover novel lasso peptides with antimicrobial activity by using DNA sequencing data to guide experimental procedures. We assessed metagenomic and bacterial whole genome sequencing data for the presence of lasso peptide gene clusters using antiSMASH5.2.0 and identified 177 potential lasso peptide gene clusters. Identified lasso peptide gene clusters were further analysed for antimicrobial potential, novelty, sequence diversity, and Actinobacterial origin. Two lasso peptide gene clusters were chosen as cloning targets for further experiments. We successfully PCR amplified one target lasso peptide gene cluster from eDNA extracted from a volcanic cave biofilm sample. Expression of the lasso peptide gene cluster was attempted by both cell-free biosynthesis and maltose-binding protein cloning. The antimicrobial potential of the obtained peptide was assessed by screening for antibacterial activity against multidrug resistant *E. coli* strains and a selection of ESKAPE pathogens. Potential inhibition of the growth of *Staphylococcus aureus* was observed. Further investigation is ongoing to confirm the antibacterial effect, the structure, and the molecule properties of the novel lasso peptide.

B165

Wee coli, engineered minicell producing E. coli for incorporating into engineered living materials

David Mark ORCID iD, Massimo Vassalli ORCID iD

University of Glasgow, Glasgow, United Kingdom

Abstract

Engineered living materials (ELMs), composed of living cells embedded in a biocompatible matrix, promise to revolutionize multiple fields including tissue engineering and artificial meat production, however there remains a challenge to precise 3D control of material properties and desired cellular outcomes. The Printed Symbiotic Living Tissues consortium (PRISM-LT) seeks to solve this problem, by developing a platform where naïve stem cells are 3D bioprinted alongside "helper" microorganisms, which are genetically modified to guide differentiation of naïve mesenchymal stem cells. The challenges facing this are two fold: control of the growth of microorganisms so that they don't outgrow and outcompete the stem cells they cohabitat with, and control of differentiation guidance to only occur in appropriate spaces.

To solve this problem, we have engineered constitutively fluorescent *Escherichia coli* K12 MG1655 which, thanks to a chromosomal *minCD* deletion, produce anucleoid minicells during their growth. We show that chromosomally encoded mBe RFP accumulates to detectable levels within these minicells to enable tracking microscopically. We test the ability of minicells to sense and respond to soluble mammalian signals using GFP-based reporter plasmids, which have been previously identified in our lab to be induced in the presence of mammalian cells. Finally, we test the ability of minicells to express and secrete synthetic growth factor fusion proteins designed by us to control stem cell differentiation.

This work highlights the challenges facing the development of ELMs, and how we known biology can be exploited to overcome those challenges.

B166

Bioaugmentation of Crops Using Bacteria Exhibiting Plant-Growth Promoting Properties.

Rebecca Morton[1], Josephine Giard[1], Ross Alexander[1], Stephen Euston[1,2], Michele Partipilo[3], Pablo Iván Nikel[3]

[1]Heriot-Watt University, Edinburgh, United Kingdom. [2]Toronto Metropolitan University, Toronto, Canada. [3]Technical University of Denmark, Kongens Lyngby, Denmark

Abstract

With climate change becoming an ever-growing threat, periods of drought stress are becoming more prevalent often leading to devastating effects on the growth, production and yield of crop plants. One potential mitigation strategy is to use plant-growth promoting rhizobacteria (PGPR) to mitigate these effects and to improve the growth and tolerance of plants under drought stress Recently, we have tested the potential of four *Pseudomonas* strains (namely *P. putida*, *P. protegens*, *P. capeferrum* and *P. fluorescens*) to see if they could improve the growth and germination of both Barley (*Hordeum Vulgare* L.) and *Arabidopsis thaliana* under osmotic stress conditions. All four strains were found to produce PGP traits including mineral solubilisation, siderophores, exopolysaccharides and enzyme production. We assessed the germination and growth of barley under osmotic stress conditions using PEG6000 and found improved germination, and that the growth of seedlings was significantly greater compared to the control when seeds were treated with *P. capeferrum* and *P. protegens*. We also tested the efficiency of culture inoculation directly into the soil of barley and *Arabidopsis thaliana* plants. We found that following a period of drought stress plants had greater shoot biomass when inoculated with *P. protegens* compared to controls (not inoculated). Furthermore, PGPR inoculation of barley significantly altered a number of physiological and biochemical responses including increased chlorophyll levels and proline. Similar effects were found in *Arabidopsis thaliana*. We are now investigating antioxidative enzyme activity and whether a combination of different strains can further improve drought tolerance.

B167

Scanning Plectasin for Improved Antimicrobial Activity

Fahimeh Masaeli, Edward Taylor, Stefan Millson

Lincoln, Lincoln, United Kingdom

Abstract

Scanning Plectasin for Enhanced Antimicrobial Properties

Antimicrobial resistance (AMR) is a global public health challenge, with the rise of multidrug-resistant bacteria emphasizing the urgent need for novel antibiotic mechanisms. Plectasin, a defensin peptide from *Pseudoplectania nigrella*, shows potential as a therapeutic agent against AMR bacteria. This study explores the antibacterial properties of both native and modified forms of Plectasin and investigates ways to enhance its effectiveness.

Mutations aimed at identifying key activity residues were generated using controlled mutagenesis methods. The antibacterial performance was assessed through minimum inhibitory concentration (MIC) and disc diffusion assays against *Bacillus subtilis 168*, with structural stability evaluated over a temperature range.

These findings highlight Plectasin's potential to interact with lipid II through critical residues, presenting a unique mechanism for bacterial membrane disruption. Structural analysis revealed that some mutations preserved essential residues, supporting further investigation into Plectasin’s therapeutic potential.

B168

Overcoming antimicrobial resistance with Plant-Based Compounds: An In Silico study of *Aegle marmelos*.

Shreyoshi Tarapdar[1], Dr.Shuai Zhang[2], Dr. Linda Oyama[2]

[1]Belfast, Belfast, United Kingdom. [2]Queen's University Belfast, Belfast, United Kingdom

Abstract

The rise of multi-drug-resistant pathogens is imposing a global concern in the field of medicine. Their interference complicates the treatment of infections followed by the increase in the mortality rate. The restricted effectiveness of currently available antibiotics, against these pathogens further increases the concern and calls for the urgent need for novel therapeutic approaches. *Aegle marmelos*, which is also commonly known as Bael, possesses a wide range of active biological compounds such as antioxidants, and alkaloids including marmesin,aegeline,marmelosin etc. However, there has been limited research done on their potential to combat multi-drug-resistant pathogens. This project aims to overcome this gap by exploring the efficacy of the secondary metabolites produced by the plant to combat the pathogens including *Salmonella* t*yphi*, *Enterococcus faecium, Neisseria gonorrhoea, Staphylococcus aureus, Candida glabrata, Histoplasma sp,* among others. With the help of computational tools such as virtual screening, toxicity prediction and molecular docking, this project will allow high-throughput selection and assessment of the selected candidates, allowing us to implement a high throughput in silico selection of lead candidates for development as alternatives to antimicrobials from this plant source.

B169

Assessing the Ecological Impacts of Microbial-based Cleaning Products on Soil Microbiomes

Deepanshi Karwall ORCID iD[1], Christopher G Knight ORCID iD[1], Andrew J Mcbain ORCID iD[1], Aline Metris ORCID iD[2], Katie Endersby[2]

[1]University of Manchester, Manchester, United Kingdom. [2]Unilever's Safety and Environmental Assurance Centre (SEAC), Colworth Science Park, Bedfordshire, United Kingdom

Abstract

Microbial-based cleaning products (MBCPs) are emerging as innovative alternatives to traditional cleaning agents, containing beneficial bacteria such as *Bacillus* species in spore form that can outcompete potential pathogens on surfaces. However, as these products gain popularity, understanding their interactions with environmental microbiomes, particularly in soils, remains critical due to the potential ecological effects of introducing additional microbial strains.

In this study, five *Bacillus* strains isolated from MBCPs were examined for growth across a pH range of 3 to 9 in an *in vitro* system. All strains demonstrated growth between pH 5 and 9, with two strains growing even at pH 4, and one showing peak growth at pH 9. Additionally, each strain was observed to adjust its surrounding pH toward neutrality, with initial pH levels from 5 to 9 converging around pH 7. Biofilm formation was observed in the two strains with the broadest pH tolerance, suggesting enhanced resilience and persistence potential. These biofilm-forming strains also exhibited inhibitory effects on other *Bacillus* strains, likely due to the production of antimicrobial compounds.

These findings contribute to understanding the ecological impact of MBCPs by defining the growth boundaries, environmental modifications, and competitive behaviour of *Bacillus* strains under varying abiotic conditions; these results inform models to predict their optimal growth conditions. Future work will investigate the implications of these interactions with soil microbial communities, ultimately supporting risk assessments for bioinoculants in natural environments.

B170

Identification and characterisation of plastic degrading microbes in UK landfills

Samuel Rattray[1], Lynsay Blake ORCID iD[2], Nia Jones ORCID iD[2], Iain MacArthur ORCID iD[1]

[1]Northumbria University, Newcastle Upon Tyne, United Kingdom. [2]Department for Environment, Food & Rural Affairs, Newcastle Upon Tyne, United Kingdom

Abstract

According to the latest National Atmospheric Emissions Inventory, 90% of greenhouse gases (GHG) emitted from the UK's waste management sector is methane, primarily from degradation of biodegradable waste in landfill. To mitigate this, the government are exploring options to work towards the near elimination of biodegradable municipal waste to landfill from 2028.

We presume currently that landfilled 'non-biodegradable' materials such as plastics don't contribute to methane emissions. Yet, plastic degrading microbes (PDMs) have been identified in landfills. Additionally, primary microplastics are disposed of and secondary microplastics are known to be generated by abiotic factors within landfills. This initial plastic deterioration can assist the depolymerisation of plastic material into absorbable products by PDMs. Potentially facilitating the metabolization of landfilled plastics into carbon dioxide aerobically, or methane anaerobically, representing an unrecognized GHG emission source.

To date, no studies have aimed to identify PDMs in UK landfills. We have analysed the leachate and bio-sludge from five UK landfill sites to characterise their phylogenetic composition and identify any known PDMs. We have combined 16S and whole genome sequencing with Oxford Nanopore Technologies to characterise the microbial communities of each sample. FTIR was used to identify and measure the types and amounts of microplastics in samples, informing selective cultivation of PDMs. Testing can then be done in plastic degradation assays to characterise metabolic activity and byproducts in similar environmental conditions as that of landfills. The results will address whether plastic waste can be degraded by the landfill microbiota, which could potentially lead to GHG emissions.

B171

Evaluating Antimicrobial Stewardship Intervention, in General Surgery Service in a District Hospital

Mai Mohamed

East and North Hertforshire, Stevenage, United Kingdom

Abstract

Background: Aiming towards decreasing the risk of antimicrobial resistance while keeping patients safe, antimicrobial stewardship emerged. The decision to use antimicrobials depends mainly on prescribers.

Methods: An audit which consists of 2 cycles was carried out in order to objectively evaluate of this stewardship. The audit and the intervention were implemented in a general surgery department.

Results: More judicious use of antimicrobials was observed after the intervention.

Conclusion: Audit and feedback antimicrobial stewardship programs can lead to drastic changes in total antimicrobial use.

B172

Exploring Host Interaction and Virulence of Atypical *Salmonella*

Amy Campbell[1], Archie Harber[1], Guerrino Macori[2], Séamus Fanning[2], Helina Marshall[1,2]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]University College Dublin, Dublin, Ireland

Abstract

Salmonella is one of the leading causes of diarrheal disease worldwide. *Salmonella* commonly resides within domestic, wild and food animals, allowing it to infiltrate the food chain and causes salmonellosis through the consumption of contaminated food. *Salmonella* serovar Typhimurium is the most common serovar isolated from foodborne outbreaks. However, factors such as global trade, climate change and globalisation has seen increased emergence of atypical Salmonellae, one being *Salmonella* Uganda. We have been working to characterise *Salmonella* Uganda and to determine its unique atypical phenotype. Initial studies have shown genetic differences in comparison to *Salmonella* Typhimurium, particularly in the Salmonella Pathogenicity Islands. This strain also showed distinct responses in infection models using *Galleria mellonella* larvae and mice. In the *Galleria* model, infections with *Salmonella* Uganda led to higher mortality rates than those caused by *Salmonella* Typhimurium. Conversely, mice infected with *Salmonella* Uganda exhibited weight maintenance or gain, greater survival rates, and an equal bacterial burden to mice infected with *Salmonella* Typhimurium. These preliminary findings suggest that emerging atypical *Salmonella* strains, such as *Salmonella* Uganda, may pose a unique threat to human health due to their novel host adaptations.

B173

Antimicrobial resistance and heavy metal tolerance in *Pseudomonas aeruginosa* isolated from different natural environments

Sreeshma Padiyath ORCID iD[1], Champa Hemachandra[2], Subbannayya Kotigadde[3], Sunil Rao Padmaraj[4]

[1]University of Central Lancashire, Preston, United Kingdom. [2]Sri Chamundeshwari Medical College Hospital and Research Institute, Karnataka, India. [3]KVG Medical College & Hospital, Karnataka, India. [4]Pushpagiri Institute of Medical Sciences and Research Centre, Kerala, India

Abstract

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium that cause infection among immunocompromised patients. It is a ubiquitous pathogen known for its metabolic versatility, adaptability and resistance to a variety of chemical compounds such as antibiotics, disinfectants and detergents, which facilitates long-term persistence in healthcare settings. Soil and aquatic ecosystems, the largest and multifarious microbial habitats on earth, could serve as storehouses of antibiotic-resistant pathogens.

The study was conducted in the Department of Microbiology, K. V. G. Medical College & Hospital, Karnataka, India. The Institutional Ethical Committee has approved the procedures used in this study. A total of 75 *P. aeruginosa* isolated from various natural environmental samples were used in the study. Antimicrobial susceptibility testing and heavy metal tolerance were done by standard procedures.

Pseudomonas aeruginosa was isolated from 54% of environmental samples. The occurrence of *P. aeruginosa* from river water (65.3%) was more compared to agricultural soil (34.7%). These isolates have demonstrated high resistance to cephalosporins, which include cefepime (70%), ceftriaxone (40%), cefotaxime (30%), ceftazidime (10%) and meropenem (2.7%). All isolates were found to be tolerant to heavy metals such as zinc, lead, copper and nickel. None of the isolates were tolerant to silver and arsenic.

This study provides insight into the propensity for antimicrobial and heavy metal resistance in *P. aeruginosa* isolated from different natural environments. The emergence of resistant bacteria in the environment poses a threat to the community. These findings demonstrate the importance of frequent monitoring of various natural environments to prevent the dissemination of these pathogens.

B174

Utilising metagenomics to investigate presumptive novel actinomycete spp isolated from the Great Salt Plains, Oklahoma.

Karen Walker[1], Paul Lawson[2], Ahmed Alhejaili[3], Alistair Brown[3], Amanda Jones[1]

[1]Northumbria University, Newcastle, United Kingdom. [2]University of Oklahoma, Oklahoma, USA. [3]Newcastle University, Newcastle, United Kingdom

Abstract

Actinomycetes have historically provided mankind with secondary metabolites that have led to the development of the majority of our antimicrobial agents, with Rifampicin and Streptomycin to name a few. The increased requirement for the discovery of new therapeutic and commercially significant antimicrobial agents has stimulated the need for the selective isolation of novel actinomycetes. In the present study, the 645 isolates (previously obtained from soil environments within the Great Salt Plains) were subjected to BOX-PCR (a repetitive sequence-based polymerase chain reaction) to group isolates according to strain differentiation, where 58 isolates were shown to be of interest within 10 distinct clades. The strains were evaluated for their ability to produce bioactive compounds against known pathogens using a modified agar plug diffusion assay.

The 58 dereplicated isolates were subjected to 16S rRNA gene sequencing to ascertain their identity and novelty within the taxonomic family, *Actinomycetales.* Results included singular isolates of *Rhodococcus, Micromonospera*, *Microbacterium, Sphingomonas* and *Pseudomonas* with the majority being *Streptomyces.* The resulting phylogenetic tree showed that 18 of the 53 *Streptomyces* isolated were presumptive novel isolates as the percentage similarity was below 98.65 but also where they branched on the tree. The pH and salt tolerance of these presumptive novel isolates were tested at pH7, pH8 and pH9 with potassium chloride and sodium chloride ranging from 0% to 15%. It was evident that some of the presumptive novel isolates tolerated a higher potassium content (up to 15%) than sodium content (up to 11%).

B175

Antibacterial effect of Garcinia kola (Bitter kola) on some clinical isolates.

Grace Adenike Akano ORCID iD

Ede, Ede, Nigeria. Bowen university, Iwo, Nigeria

Abstract

This study was conducted to test for the Antibacterial effect of Garcinia kola (Bitter kola) on some clinical isolates. Bitter kola seeds were purchased from dealers at Oja Timi Market, Ede, Osun State Nigeria and brought to the Microbiology laboratory of Federal Polytechnic Ede, Osun State for laboratory analysis. The Garcinia seed (nut) were dehusked cut into pieces, air-dried and grinded into powdered form. 10g of powdered nut was subjected to extraction using different solvents in 100ml such as 95 % aqueous ethanol, cold and hot water at room temperature for 24 hours and filtered with sterile filter paper. Five clinical isolates (Staphylococcus aureus, Proteus mirabilis,Escherichia coli, Klebsiella pneumoniae and Streptococcus pneumoniae) cultured from the throat were gotten from UNIOSUN Teaching Hospital microbiology laboratory and was screened. The isolates were tested for sensitivity using MacConkey agar by culturing them at 370C for 24hours. The result of the sensitivity test revealed that the Ethanol extract (100 mg/ml) yielded the highest zones of inhibition followed by Hot water extract then cold water extract against Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Klebsiella pneumonia and Streptococcus pneumonia with the following diameters of zones of inhibition respectively:(21mm, 18mm, 20mm, 18mm, and 20mm); (16mm, 13mm, 16mm, 14mm, and 15mm); (13mm, 8mm, 13mm, 11mm, and 12mm).The bitter kola extract was thus revealed to be best extracted with ethanol solvent as it enhances the bioactive compound or component of the plants. Antibiotic sensitivity test was also carried out on the clinical isolates with some of the standard antibiotics commercially sold for the treatment of throat disease and the following result was obtained; Amoxicillin was reactive against E. coli, K. p and S. p (7mm, 4.5mm and 24mm) respectively but did not react against S. a and P m. Erythromycin reacted against S. a and S. p (22mm and 6mm) respectively.But did not react against P. m, E. c and K. p. Azithromycin reacted against S. a, P. ms, E. c and K. p (16mm, 24mm, 15mm and 20mm) respectively but was unreactive against S. p. Streptomycin was reactive against only S. a (10mm) and unreactive against P. m, E. c, K. p and S. p. Ciprofloxacin reacted on all the five (5) clinical isolates S. a, P. m, E. c, K. p and S. p (20mm, 21mm, 17mm, 23mm and 15mm respectively). Lastly, Ampicillin was reactive against P. m, E.c and S. p (11mm, 9mm and 21mm respectively). The antibacterial activity of the bitter kola was therefore compared with the standard antibiotics and it was revealed and recommended that Garcinia kola extracts could be used by the pharmaceutical industries to produce drugs that can cure or prevent throat infections.

B176

Antibacterial and Antifungal Effect of Essential Oils from cinnamon leaf (Cinnamomum zeylanicum), oregano (Origanum vulgare) and thyme (Thymus vulgaris)

Behnaz Azimzadeh[1], Muiz Akinyemi[2], Bukola Onarinde ORCID iD[1]

[1]University of Lincoln, Holbeach, United Kingdom. [2]university of Leeds, leeds, United Kingdom

Abstract

Recently, there has been a growing interest in essential oils (EOs) for their potential as natural antimicrobial and antioxidant agents. This trend is largely driven by a shift in consumer attitudes against synthetic additives and a rising demand for innovative functional foods that offer potential health benefits.

The aim of this study was to identify and characterise the key components of EOs from cinnamon leaf (*Cinnamomum zeylanicum*) (EOC), oregano (*Origanum vulgare*) (EOO) and thyme (*Thymus vulgaris*) (EOT) essential oils and evaluate by *in vitro* study their antimicrobial potential.

Key component of each EO was determined using GC-MS technique. Each EO was screened against important pathogens that pose significant challenges due to their roles in foodborne illnesses, antibiotic resistance, and their ability to form resilient biofilms. Using the agar well diffusion method EOC, EOO and EOT were tested against *Escherichia coli, Enterococcus faecium*, *Aspergillus Niger* and *Pseudomonas*.

GC-MS analysed identified eugenol as the major compound in EOC (70.4%), thymol in EOT (29%), and carvacrol in EOO (93%). Additionally, EOC was rich in caryophyllene, linalool, β-phellandrene, and copaene; EOT contained significant terpinolene; and EOO was rich in p-cymene, γ-terpinene, thymol, β-pinene, and D-limonene. The results obtained from the *in vitro* study revealed that these EOs exhibited strong (clear inhibition zones) antimicrobial properties against the tested pathogens.

These findings suggest that the active compounds in EOC, EOO and EOT hold promise as effective and economical natural antimicrobial agents, with potential applications in various setting including food, health and environmental settings.

B177

A comparative study of the growth of Pseudomonas fluorescens and other target organisms of interest in Traditional Camembert and Plant-based Cheese

Rose Mante[1], Oluyemi Awolusi[1], Enrico Ferrari[2], Bukola Onarinde ORCID iD[1]

[1]University of Lincoln, Holbeach, United Kingdom. [2]University of Lincoln, Lincoln, United Kingdom

Abstract

Plant-based food have gained remarkable interest fuelled by the increasing interest in sustainable environmental practices, consumer preferences towards healthy and ethically mindful food choices. Although studies have revealed diverse microbial communities in plant-based food with potential implications for storage strategies and product shelf-life there is limited data on ability of these novel products to support microbial growth.

This study aimed to investigate growth of *Pseudomonas fluorescens* and other natural flora in plant-based and traditional cheese stored at different storage temperatures. Traditional and plant-based camembert-style cheeses were inoculated with *P. fluorescens* and stored at 4°C and ambient temperature. The growth and presence of *P. fluorescens*, lactic acid bacteria, total aerobic bacteria and yeast and moulds were studied over the storage period.

After 10 days both samples stored at room temperature showed higher microbial numbers as compared to those stored at 4°C.

A measure of the Area Under the Curve (AUC) for comparing bacterial growth over time indicated a significant difference between bacterial growth across the different storage temperatures.

A measure of colour for traditional cheese indicated that there was darkening of the cheese when stored at 4°C however the cheese colour was observed to become lighter at room temperature. However, this was not observed for the plant-based cheese. Both cheese types developed yellow and green tones as storage time progressed.

The data gathered helps us understand how different cheeses respond to temperature changes in terms of bacterial growth, crucial for determining optimal storage conditions for food safety and quality.

B178

Selective silencing: uncovering how the *Pseudomonas aeruginosa* signal HHQ tunes into some *Vibrio* species and gets tuned out of others

Conor Hill[1], Ana Ramos[2], Claudia Serra[3], Nicky O'Boyle[4], F. Jerry Reen[1,5]

[1]University College Cork, Cork, Ireland. [2]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal. [3]CIIMAR, - Centro Interdisciplinar de Investigação Marinha e Ambiental University of Porto, Porto, Portugal. [4]Trinity College Dublin, Dublin, Ireland. [5]Synthesis and Solid-State Pharmaceutical Centre, University College Cork, Cork, Ireland

Abstract

The chemical diversity evident in the bioactive potential of marine microbes has yet to be reflected in the range of chemical signals controlling behaviour and colonisation in this important natural ecosystem. Emerging as a treasure trove of untapped bioactive potential, it is somewhat surprising that very little is known about the scope of chemical communication within marine microbial communities. An intriguing species-specific interplay has emerged between two genera found within the marine sponge microbiome, *Pseudomonas* and *Vibrio*.

Along with its precursor HHQ, the Pseudomonas Quinolone Signal (PQS) is known to play an important role in virulence and biofilm formation in *P. aeruginosa*. HHQ has emerged in its own right as an interkingdom signal, targeting biofilm formation in bacterial and fungal pathogens. It has also demonstrated species-specific antibacterial activity against members of the *Vibrionaceae*, with even minor alterations in the structure of the signalling molecule resulting in complete loss of inhibition.

A combination of strain and species level inhibition studies, allied with comparative genomics analysis, revealed several candidates for HHQ tolerance. Initial efforts focused on the *V. parahaemolyticus* encoded *cydAB* genes. However, mutation of these genes did not alter the tolerance phenotype. Enzymatic protection from the action of HHQ has emerged as a potential mechanism, with supernatants of *V. parahaemolyticus* able to disrupt the antibacterial activity of HHQ. Though best known as human pathogens, understanding the dynamics of these interspecies interactions will provide important insights into the microbiome population shifts that underpin marine sponge colonisation, particularly those controlled by quorum sensing.

B179

Bioprospecting Arthrobacter and Pseudarthrobacter spp. from Marine Ecosystems for Novel Antimicrobial Compounds

Fairouz S. H. Gzara, Stephen A. Jackson, Alan D. W. Dobson, David J. Clarke

School of Microbiology, University College Cork, Cork, Ireland

Abstract

With the rising prevalence of multi-resistant microbial strains, the pursuit for novel antimicrobial compounds has become urgent. Through the MARBLES project, an EU initiative involving 14 partners across Europe, we are investigating commensal microorganisms from diverse marine ecosystems as potential sources of bioactive molecules. Initial genomic analyses of nine isolates from the skin microbiome of Atlantic salmon (*Salmo salar*) identified *Arthrobacter* and *Pseudarthrobacter* species as promising candidates for novel bioactive compound biosynthesis. Building on these findings, our study has expanded to include 25 *Arthrobacter/Pseudarthrobacter* isolates sourced from healthy salmon skin, marine sponges (*Raspailia ramosa*, *Stelligera stuposa*, *Leucosolenia* sp.) and additional marine habitats (including sea ice, meltwater, surface seawater, deep-sea seawater and copepods). To evaluate the antimicrobial potential of these isolates, we have performed overlay assays to assess bioactivity against pathogens and analysed whole genome sequencing data to assess biosynthetic gene clusters' potential for novel bioactive compounds production. Additionally, we utilized Biolog Odin III phenotypic profiling to uncover functional diversity within this collection. Our approach highlights the untapped diversity and bioactivity within *Arthrobacter/Pseudarthrobacter* spp. from marine environments, underscoring their potential as sustainable alternatives to traditional antibiotics in aquaculture.

B180

Soil Microbiome and Bacterial Consortium: Keys to Sustainable Potato Soil Management

Paula V. Morais[1], Adriana Mata[1], Carlos Cruz[2], Marta Acin-Albiac[3], Beatriz Garcia-Jimenez[3], Alberto Acedo[3], Isabel Conceição[2], Diogo Neves Proença[1,4]

[1]University of Coimbra, Department of Life Sciences and CEMMPRE, ARISE, Coimbra, Portugal. [2]University of Coimbra, Department of Life Sciences and CFE, TERRA, Coimbra, Portugal. [3]Biome Makers Inc., West Sacramento, USA. [4]MED & CHANGE, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Faro, Portugal

Abstract

Solanum tuberosum **represents a vital component of global agriculture, a staple food source for millions. However, the** low potato yield average in European countries may be attributed to plant-parasitic nematodes(PPN).

This work aims to i) assess the microbiome of potato fields of healthy soils(without PPN) and diseased soils(with PPN) to understand the effect of PPN on soil microbial diversity and ii) find the beneficial bacterial consortium for effective management of PPN and plant growth-promotion (PGP).

A total of 450 soil samples from 45 fields were collected from the North to the South of Portugal. The microbiomes and nematodes were identified before(T0) and after(T1) potato cultivations. Moreover, 30 bacterial strains were isolated and characterized regarding PGP traits, antibiotic resistance, nematicidal activity, and plant phytosanitary.

The α-diversity was significantly higher in T1 and the presence of PPN significantly increased diversity indices for bacterial and fungal communities. Organic management had a significantly higher median Soil Quality Index than conventional management. *Bacillus subtilis* was the most abundant species in organic management. As part of the core microbiome, it was found the saprophytic genus *Humicola*. The bacterial consortium was established as a biological and sustainable alternative for chemical reduction practices, and it was composed by one *Bacillus* and two *Pseudomonas* strains showing complementary PGP traits and 100% efficacy on nematodes mortality.

In conclusion, this study contributes to a better overview of organic and conventional management and shows that the use of beneficial microorganisms can be a real opportunity for a more sustainable agriculture.

Session : Infection Forum

B181

The use of Matrix Assisted Laser Desorption Ionisation time of flight (MALDI-TOF) for the detection of Methicillin Resistant, Panton Valentine Leukocidin (PVL) and Toxic Shock Syndrome Toxin 1 (TSST-1) positive Staphylococcus aureus

Natalie Lamont ORCID iD, Lewis Bingle, Callum Cooper

University of Sunderland, Sunderland, United Kingdom

Abstract

Introduction

Staphylococcus aureus (S. aureus), a common skin bacterium, causes a variety of human diseases, through production of several exotoxins and other secreted virulence factors, including Toxic Shock Syndrome Toxin-1 and Panton-Valentine leucocidin (PVL). Methicillin resistant *S. aureus* (MRSA) is harder to treat, associated with increased morbidity and mortality and of particular concern within hospitals worldwide. Infections caused by *S. aureus* possessing these toxins or resistance mechanisms are burdens on healthcare in the UK

Method

PVL and TSST production was assessed using nutrient broth liquid medium, tryptone soy broth liquid medium, CCY. Bacteria was cultured in each media and analysed using western blotting to determine the most suitable culture method.

MALDI-TOF analysis of known *S. aureus* PVL, TSST and MRSA positive isolates using NCTC strains and patient positives isolates using whole cell, partial extraction, full extraction, culture supernatant and protein precipitation methods from the range of media.

Results

TSB allowed for protein production and CCY increased protein production. MALDI-TOF data shows no difference between spectra of different strains from whole cell, liquid media, protein extraction procedures and expansion of them/z range analysed. Next steps are to analyse spectra produced using different matrices and method optimisation for MALDI-TOF to refine spectra and background noise.

Conclusion

This study aims are detection of PVL, MRSA and TSST-1 in clinical isolates using MALDI-TOF. If successful, these approaches could be implemented into existing diagnostic workflows, reducing the time to

detection, allowing enhanced infection prevention and control measures, and reducing hospital acquired infections.

B235

Identification of a small molecule BFstatin inhibiting BrpR, the transcriptional regulator responsible for *Vibrio vulnificus* biofilm development

Hojun Lee, Dayoung Sung, Gaeun Lee, Sang Ho Choi

Seoul National University, Seoul, Korea, Republic of

Abstract

Many pathogenic bacteria form biofilms that are resistant to not only host immune defenses but also antibiotics, posing a need for the development of strategies to control biofilms. In this study, to prevent biofilm formation of the fulminating foodborne pathogen *Vibrio vulnificus*, chemical libraries were extensively screened to identify a small molecule inhibiting the activity of BrpR, a transcriptional regulator for biofilm genes. Accordingly, the BrpR inhibitor BFstatin [N1-(2-chloro-5-fluorophenyl)-N3-propylmalonamide], with a half-maximal effective concentration of 8.01 μM, was identified. BFstatin did not interfere with bacterial growth or exhibit cytotoxicity to the human epithelial cell line. BFstatin directly bound to BrpR and interrupted its binding to the target promoter DNAs of the downstream genes. Molecular dynamics simulation of the interaction between BFstatin and BrpR proposed that BFstatin modifies the structure of BrpR, especially the DNA-binding domain. Transcriptomic analyses revealed that BFstatin reduces the expression of the BrpR regulon including the *cabABC* operon and *brp* locus which contribute to the production of biofilm matrix of *V. vulnificus*. Accordingly, BFstatin diminished the biofilm levels of *V. vulnificus* by inhibiting the matrix development in a concentration-dependent manner. Altogether, BFstatin could be an anti-biofilm agent targeting BrpR, thereby rendering *V. vulnificus* more susceptible to host immune defenses and antibiotics.

B236

Severity and its associated factor among patients admitted to karl comprehensive specialized hospital Mattu, oromia, Ethiopia. Retrospective facilitate based crosectional study.

Lijalem Kassa Yehuale

Gambella General Hospital, Gambella, Ethiopia

Abstract

Method: A facility based retrospective cross sectional study was conducted from May 15, 2023 to Aug 15, 2023 and one-year data was retrieved from May 2021 up to May 2020. A simple random sampling technique was employed to include a total of 404 sample populations in the study. A standardized questionnaire was used to collect relevant raw data. Finally the raw data was checked manually for completeness, then coded and the template was prepared and entered into Epi-data V.3.1 statistical software and cleaned thoroughly before exported to SPSS version 20 for further analysis. The descriptive statistics was calculated & ordinal logistic regression analysis was used to see the relation between dependent variable and independent variables. The association was assessed by using the Chi-Square statistical test method (X2–test) and P value <0.05 was considered as statistically significant. The assumption of model fitness, parallel tastes was fulfilled and Multicollinearity was checked, the VIF was less than 10.

Result: 86.8%, 9.5%, and 3.8% of the 404 patients who are quarantine at the facility were mild, moderate, and severe, respectively. Live in urban is the common significant factors. Fever, Coughing and headaches were the most typical signs and symptoms linked to the disease's severity. The most frequent co-morbidity was hypertension.

B237

THE ACTIVITIES OF SUAVEOLOL AND OTHER COMPOUNDS FROM HYPTISSUAVEOLENS AND MOMORDICA CHARANTIA AGAINST THE AETIOLOGICALAGENTS OF AFRICAN TRYPANOSOMIASIS AND MALARIA

Enimie Oaikhena ORCID iD[1], Nkechi Egbe ORCID iD[1], Yahaya Umar[1], Harry Dekoning[2]

[1]Nigerian Defence Academy, Kaduna, Nigeria. [2]University of Glasgow, Glasgow, United Kingdom

Abstract

African trypanosomiasis and malaria are among the most severe health challenges to humans and livestock in Africa and new drugs are needed. Leaves of *Hyptis suaveolens* and *Momordica charantia* were extracted and subjected to silica gel column chromatography. Structures of isolated compounds were elucidated through NMR and HR-EIMS spectrometry. The isolated biomolecules were tested against trypomastigotes of *Trypanosoma brucei brucei, T. congolense*, and *Plasmodium falciparum*. Callistrisic acid, dehydroabietinol, suaveolic acid, suaveolol, and a mixture of suaveolol and suaveolic acid were obtained from *H. suaveolens*, while karavilagenin D and momordicin I acetate were obtained from *M. charantia*. The most promising EC_{50} values were obtained for suaveolol alone at 2.7 1± 0.36 µg/ml, or in a mixture together with suaveolic acid, exhibiting an EC_{50} of 1.56 ± 0.17 µg/ml against *T. b. brucei* trypomastigotes. Suaveolic acid alone had low activity against *T. b. brucei* but displayed moderate activity against *T. congolense* trypomastigotes at 11.1 ± 0.5 µg/ml. Moderate activity was displayed by suaveolol against *Plasmodium falciparum* while SSA exhibited potent activity with an EC_{50} of 2.10 ± 1.40 µg/ml. Neither of the active compounds nor the mixture of the two, displayed any cytotoxic effect on human foreskin fibroblast (HFF) cells at even the highest concentration tested, being 200 µg/ml. We conclude that suaveolol and its mixture possessed significant and selective trypanocidal activity.

B238

Antimicrobial Activity of Cannabinoids Towards Common Equine Wound Pathogens and their Biofilms

Maria Júdice da Costa, Debbie Nash, David Whitworth, Ruth Wonfor

Aberystwyth University, Aberystwyth, United Kingdom

Abstract

Wounds are a major welfare problem and the second most common cause of death/euthanasia in horses. Equine wounds often heal poorly and are prone to infections, which are typically treated with antimicrobials. However, rising antimicrobial resistance is a global concern. Cannabinoids, in particular cannabidiol (CBD) and cannabigerol (CBG), have shown potential as antimicrobials, particularly against Gram-positive bacteria. This study aimed to test the antimicrobial activity of crude hemp extract (CE), CBD, and CBG against common equine wound pathogens and their biofilms, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, and *Enterococcus faecalis*. The antimicrobial activity of the cannabinoids was determined using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays, while biomass quantification assays were used to determine whether cannabinoids prevented biofilm formation. CBD and CBG were effective against Gram-positive bacteria with MICs ranging from 2.5 to 40 µg/mL, compared to antibiotic control MICs ranging from 0.15 to 80 µg/mL. None of the tested cannabinoids showed antimicrobial activity against Gram-negative bacteria and CE had no effect on any of the tested bacteria. CBD and CBG prevented biofilm formation of Gram-positive bacteria at similar concentrations to their MIC values. CBD and CBG were bacteriostatic against *B. subtilis* and *E. faecalis* and had a bactericidal effect on *S. aureus* and *S. epidermis*, with MBCs ranging from 10 to 20 µg/mL. Further work is ongoing to test cannabinoids' antimicrobial activity on more equine wound pathogens and to test if cannabinoids can disrupt pre-formed pathogen biofilms.

B239

How realistic can these lung models be when testing the attenuation of the ORF10KO virus?

Shichun Gu[1], Erly Raras Savitri[1], Isobel Webb[1], Parul Sharma[2], Ellie Bentley[2], Adam Kirby[2], Dan Mega[2], James Stewart[2], Wael Kafienah[1], Andrew Davidson[1], David Matthews[1]

[1]University of Bristol, Bristol, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom

Abstract

The last proposed gene, open reading frame 10 (ORF10), of the SARS-CoV-2 genome does not have any direct homologs in previous pathogenic human betacoronaviruses, such as SARS-CoV or MERS-CoV. We made an ORF10 knock-out virus (ORF10KO, mutating both methionines to stop codons) in a Wuhan wild-type (WT) background. The growth characteristics of the ORF10KO virus are similar to those of the WT control virus in various carcinogen cell lines (such as Vero, A549 and Calu-3). However, the ORF10KO virus was significantly attenuated in the hamster model of SARS-CoV-2 infection. While both viruses replicated to similar titres in the nasal cavity, there was a 10-fold titre reduction in the lungs.

To further unpick the infection characteristics of ORF10 in SARS-CoV-2 pathogenesis, we employed three primary cell models of increasing complexity for infectivity analysis. The first being primary pneumocytes, a mixture of alveolar type 1 and type 2 cells. The second model is low passage primary human cells growing as air-liquid interface cultures. Alveolar epithelial cells grow on the apical side of a porous membrane, while endothelial cells grow on the basal side and have contact with the culture medium. The third and the most complex model is induced pluripotent stem cell (iPSC)-derived human lung organoids. This model generates alveolar progenitors and airway-like mesenchyme-surrounding structures. Our analysis of viral growth in these three models suggests notable differences in the replication of the ORF10KO virus compared to WT.

B240

Staphylococcus aureus and *Pseudomonas aeruginosa* co-infection in an *ex-vivo* model of cystic fibrosis lung infection

Phoebe Do Carmo Silva[1], Darryl Hill[2], Freya Harrison[1]

[1]University of Warwick, Coventry, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

Staphylococcus aureus and *Pseudomonas aeruginosa* are the most common pathogens cultured together in the lungs of people with cystic fibrosis (CF), and co-infection is often associated with poor prognosis. However, how these pathogens survive together *in vivo* is poorly understood, as *S. aureus* is often killed by *P. aeruginosa in vitro*.

To elucidate the mechanisms of co-infection, I am using an *ex-vivo* pig lung model (EVPL) as a high validity model for respiratory infections, combined with synthetic CF sputum media (SCFM) to mimic the nutritional composition of CF sputum. Using this model, and an array of CF clinical isolates, I aim to answer key questions about how *S. aureus* and *P. aeruginosa* co-infection affects CF lung disease progression including:

- What is the biofilm structure during co-infection versus single infection on lung tissue?
- Do virulence factors differ during co-infection versus single infection?
- Does prophylactic treatment of *S. aureus* in the lung alter subsequent *P. aeruginosa* colonisation?
- What is the transcriptomic analysis of *P. aeruginosa* in the presence of *S. aureus* compared to single culture?

I have found through cross-streaking on SCFM agar, clinical isolates of *P. aeruginosa* are less inhibitory towards *S. aureus* isolates cultured from the same patient compared to laboratory strains. I am then using selected paired isolates to investigate their viability, biofilm structure, phenotypes and virulence when grown in the EVPL model. These results will be used to gain a better understanding of the driving factors of *S. aureus* and *P. aeruginosa* co-existence in the CF lung.

B241

A case of multifocal myositis following a Campylobacter jejuni gastrointestinal infection

Konstantinos Teopoulos Lamprianidis ORCID iD, Orla Forker, Ashley David Price

Department of Infection and Tropical Medicine, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle Upon Tyne, United Kingdom

Abstract

Introduction

Campylobacter infection, one of the most prevalent gastroenteritis causes worldwide, commonly follows ingestion of contaminated undercooked poultry. Extra-intestinal complications, such as Guillain-Barré syndrome and reactive arthritis, are frequently reported. Myositis, however, is an extremely rare association. We present a multifocal myositis case following confirmed Campylobacter infection.

Case description

A previously well 17-year-old female presented ten days after consuming chicken at a restaurant. The initial four-day febrile diarrhoeal illness resolved and three days later she developed flank and central abdominal pain, red-coloured urine and myalgia over her back and limbs, worse over the thighs. She denied excessive exercise and taking any medications or illicit drugs.

On admission, examination revealed limb and abdominal muscle tenderness with difficulty standing. Creatine kinase exceeded 100,000U/L. Between the second and sixth day, troponin peaked at 174ng/L, ALT at 554unit/L, and AST at 1287unit/L. The autoimmune screen and urine toxicology were negative. Femur MRI revealed muscle oedema affecting multiple muscles in legs and back, consistent with inflammatory myositis. Electrocardiogram, echocardiograph, cardiac MRI, and CT abdomen were unremarkable. Campylobacter jejuni was initially identified by PCR before being isolated in faecal cultures. She was diagnosed with secondary post-infectious myositis.

She received a five-day azithromycin course and intravenous fluids. The presumed myocarditis was treated with a three-day ibuprofen course and a three-month colchicine course.

Conclusion

Campylobacter-associated myositis is an extremely rare presentation. In this case, the prompt diagnosis and early treatment initiation led to full resolution. The aim of this case report is to raise awareness about this presentation.

B242

Hypoxic niches in the cystic fibrosis lung shape both the initial colonisation and chronic infection establishment of *Pseudomonas aeruginosa*.

Joanna Drabinska ORCID iD[1,2], Lucia O'Connor[1,2], Niamh Duggan ORCID iD[1,2], Siobhán McClean ORCID iD[1,2]

[1]School of Biomolecular and Biomedical Sciences, University College Dublin, Dublin, Ireland. [2]UCD Conway Institute of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland

Abstract

Pseudomonas aeruginosa is an opportunistic bacterial pathogen causing chronic lung infections in people with cystic fibrosis (CF), contributing to morbidity and mortality. However, the mechanisms promoting adaptation towards chronic infection in the CF lung are not well understood. This work examines the impact of low oxygen (a key environmental pressure in the CF lung) on *P. aeruginosa* to determine its role in initial colonisation and persistence.

After 28-day exposure to 6% oxygen, two distinct *P. aeruginosa* small colony variants (SCVs) emerged, one exclusively in hypoxia. Importantly, SCVs were more prevalent in hypoxia-adapted cultures than in normoxia-adapted cultures (98% vs <35%). Proteomic analysis revealed significant changes in the abundance of >200 proteins, including those associated with antibiotic resistance, iron acquisition and biofilm formation. Hypoxia-adapted cultures developed higher resistance to 8 of 13 antibiotics tested; increased biofilm (1.8 to 4.1-fold ($p<0.0001$)) and decreased pyocyanin production (2.6-fold, $p<0.0001$). All hypoxia-adapted cultures showed decreased siderophore production (3.6 to 2.9-fold, $p<0.02$).

Expression of >400 genes was altered in hypoxia-exposed *P. aeruginosa* within 2 hours, with 95 upregulated. L-lactate metabolism associated genes (*lldA*, *lldP*, *lldDE*) showed dramatic induction (9.4 to 27.9-fold), and since L-lactate plays an important role in macrophage survival and virulence, this may be crucial for early CF-lung colonisation. Additionally, *PA5446*, associated with oxidative stress resistance, was induced 12.5-fold, while a holin-antiholin-like system increased 8.3-fold.

Overall, this confirms that hypoxic niches in the CF lung initially benefit *P. aeruginosa* colonisation and may facilitate the switch from acute to chronic infection in the lung.

B243

Oxidative stress and low relative humidity drive the loss of viability in airborne *Neisseria meningitidis*

Mia Dierks-Treece, Robert Alexander, Jonathan Reid, Allen Haddrell, Adam Finn, Darryl Hill

University of Bristol, Bristol, United Kingdom

Abstract

Despite advancements in vaccine development for preventing meningococcal disease, the transmission of *Neisseria meningitidis* is poorly defined, particularly in the aerosol phase.

As the polysaccharide capsule of *N. meningitidis* is important for survival in the blood, we aimed to examine its protective role in the aerosol microenvironment. Using CELEBS (Controlled Electrodynamic levitation and extraction of bioaerosol onto a substrate) we measured bacterial viability of pairs of acapsulate and encapsulated strains of Serogroup A (C751-derived) and B (MC58-derived) post-aerosolization in artificial saliva.

At 30 and 55% RH, the encapsulated C751 strain maintained significantly greater viability than its acapsulate counterpart. Both MC58 and C751 strains also exhibited decreasing viability as humidity decreased. The antioxidants thiourea and glutathione significantly increased airborne viability in encapsulated MC58, as previously shown for *E. coli.*

These findings suggest the C751 capsule provides a structural-specific advantage in aerosol. We have also evidenced the role of oxidative stress in reducing aerosol viability, furthering our understanding of the mechanisms that drive loss of viability post-aerosolisation.

Further research will explore survival in different serogroups of *N. meningitidis,* to determine if there are strain-specific survival advantages, and will use real saliva samples to understand bacterial-saliva interactions in aerosol, and therefore how these may be involved in dissemination.

B244

Loss of a functional electron transport chain promotes the uptake and intracellular persistence of *Staphylococcus aureus* in neutrophils

Elizabeth Ledger[1], Mario Recker[2], Ruth Massey[1,3]

[1]University College Cork, Cork, Ireland. [2]University of Exeter, Exeter, United Kingdom. [3]University of Bristol, Bristol, United Kingdom

Abstract

Staphylococcus aureus is a leading cause of bloodstream infections, causing over 12,500 cases of bacteraemia each year in the UK. To do this, *S. aureus* needs to be able to survive the hostile conditions of the bloodstream, including exposure to immune cells such as neutrophils. The exact mechanisms used by *S. aureus* to survive in the bloodstream remain to be elucidated, with studies suggesting that they can adopt more than one pathogenic route to achieve this. To understand this in greater detail, we used a population-based approach to determine the bacterial factors that contribute to neutrophil uptake and intracellular survival. Amongst a large collection of bacteraemia isolates we found that over 5% of strains had a single nucleotide polymorphism in their protoheme IX farnesyltransferase-encoding gene, *cyoE.* We demonstrate that this reduces the activity of their electron transport chain and leads to these strains being more efficiently phagocytosed by neutrophils. This increased uptake is due to an accumulation of intracellular fatty acids causing downregulation of the gene encoding the immunoglobulin-binding protein Sbi, a key immune evasin. However, once inside the neutrophils, these strains not only show enhanced survival but are able to grow due to their increased resistance to reactive oxygen species. That these bacteria with dysfunctional electron transport chains have been isolated from bacteraemic patients suggests that this ability to use neutrophils as an intracellular niche provides a selective advantage in this environment, and demonstrates yet another route through which this highly versatile pathogen can cause invasive disease.

B245

Evaluating a library of antimicrobial cationic polymers: from antimicrobial activity to development of resistance.

Natasha Reddy, Charlotte Cornbill, Freya Harrison, Sebastien Perrier

University of Warwick, Coventry, United Kingdom

Abstract

The development of novel antimicrobials to treat biofilms is urgently required. Biofilm formation plays a significant role in bacterial persistence and resistance to antibiotics, with up to 80% of bacterial infections being linked to biofilm forming bacteria. By utilising polymer chemistry, specifically using reversible-addition fragmentation chain transfer (RAFT) polymerisation, we can synthesise amphiphilic polymers containing cationic and hydrophobic functional units. Previous work has established these polymers as a promising platform for the development of novel biofilm-active antimicrobials. In the present work we synthesised and evaluated four ammonium-containing polymers. Their antimicrobial activity varied dependent on structure and size of the polymer, but one polymer displayed potent activity against all Gram-negative bacteria tested (*Pseudomonas aeruginosa, Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae*). While not all polymers demonstrated a strong antimicrobial effect against *P. aeruginosa* in planktonic MIC assays, all were able to reduce both biofilm biomass and eradicate viable *P. aeruginosa* in a Calgary device. Polymer-antibiotic combinations also demonstrated synergy in checkerboard assays. Finally, to determine whether resistance readily evolves against the polymers, an evolution experiment was conducted using 6 parallel populations of *P. aeruginosa.* Analysis of clones isolated at early and late timepoints demonstrated that limited resistance evolved, with a maximum of a 2-fold increase in MIC. We show that compounds with poor antimicrobial activity against planktonic cells can be effective antibiofilm agents, particularly in reducing biofilm biomass. Further, these compounds can enhance the activity of antibiotics against planktonic bacteria. Additionally, the evolution of resistance against the most active polymer is limited.

B246

Prevalence of Methicillin-Resistant Staphylococcus Aureus Among ICU Nurses In A Private Tertiary Hospital In Cebu City

Randolph Libres, Leo Anne Hao

Cebu Doctors University Hospital, Cebu, Philippines

Abstract

Background:

Antimicrobial resistance (AMR) is a global crisis, with 1.27 million deaths directly linked to it in 2019 and 4.95 million more deaths associated. Methicillin-resistant Staphylococcus aureus (MRSA) is especially problematic in healthcare, increasing both patient mortality and hospital costs. The Philippines reported 1,949 MRSA cases in 2022, mostly community-acquired. Healthcare workers (HCWs) are frequent carriers, and MRSA is easily spread in hospitals. This study investigates the prevalence of MRSA among ICU nurses to improve infection control practices.

Objectives:

The study aims to determine MRSA prevalence among ICU nurses in a Cebu hospital as of July 2024. It will assess nasal MRSA carriage, analyze phenotypic characteristics, and use the BD Phoenix system for MRSA detection, ultimately suggesting improvements to infection control protocols.

Methods:

A cross-sectional design will evaluate MRSA nasal carriage among 26 ICU nurses. Methods include blood agar culture, gram staining, catalase and coagulase tests, and automated identification via the BD Phoenix system. Results will be analyzed descriptively.

Results and Discussion:

MRSA prevalence was 7.7%, aligning with global data. All 26 isolates were Gram-positive cocci; 73.1% were catalase-positive, confirming Staphylococcus. Of these, only 7.7% were coagulase-positive, identifying MRSA. Beta-hemolysis was observed in all MRSA strains, indicating higher virulence.

Conclusion:

With a 7.7% MRSA prevalence among ICU nurses, enhanced surveillance and infection control are essential to reduce MRSA transmission in ICU settings.

B247

Systematic review and meta-analysis of the antimicrobial activity of a natural remedy against bacterial infections

Samreen Amjad, Blessing Anonye

Department of Life Sciences, Manchester Metropolitan University, Manchester, United Kingdom

Abstract

Introduction: New antimicrobials are needed to fight antibiotic-resistant organisms. Natural remedies have been shown to have antimicrobial potential. This study aimed to provide evidence for ginger's antibacterial activity as a novel antimicrobial source.

Methods: The systematic review was performed following the guidelines by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. Searches for primary research assessing the antimicrobial nature of ginger were conducted using PubMed, Scopus, Web of Science, and the Cochrane database of controlled trials. The inclusion criteria were articles reporting the antibacterial activity of ginger by minimum inhibitory concentration (MIC) or zone of inhibition (ZOI). Articles that did not focus on antibacterial activity or combinations of ginger with other natural products were excluded.

Results and Discussion: A total of 1993 articles were retrieved; 610 duplicates were removed, and the titles of 1383 articles were screened. After applying the exclusion criteria, 147 full-text articles were assessed for key quality parameters such as clear experimental protocols detailing the assay and media used, the number of replicates and controls. Overall, 20 papers were deemed eligible for further analysis. Ginger extracts were prepared using various solvents such as ethanol, methanol, hexane, ethyl-acetate and water. The antibacterial activity of ginger depended on the extraction solvent and concentration used. Overall, for studies that included the results of the positive control, the bioactivity of ginger was similar to that of the controls. More studies with detailed controls are needed to ascertain the antibacterial activity of ginger.

B248

Cysticercosis/*T. solium* taeniasis, a Potential Public Health Concern in Non-Endemic Country, Kuwait: A New Diagnostic Method to Screen *T. solium* Taeniasis Carriers Among the Expatriate Population

JAMSHAID IQBAL

College of Medicine, Kuwait University, Jabriya, Kuwait

Abstract

Objective: Kuwait is non-endemic for *Taenia solium* infection due to strict restriction on pig-farming and pork consumption however, several cases of cysticercosis were detected among Kuwaiti nationals with no history of travel to endemic countries. Infected domestic helpers/food handlers from endemic countries who may have escaped detection of infection by microscopy on their entry to Kuwait were suspected as the possible source of infection. This study determined the seroprevalence of *T. solium* among domestic helpers/food handlers by using a sensitive taeniasis-specific anti-rES33 antibody assay. ***Subjects and Methods:*** Newly arriving domestic helpers (n = 500) and food handlers (n = 500) from endemic countries were enrolled during 2015-2017. *T. solium*-specific rES33 antigen was expressed and purified from human embryonic kidney (HEK) 293-6E cells using pTT5 mammalian expression vector. Stool samples were processed for microscopy, and blood samples were screened to detect anti-*T. solium* taeniasis-specific IgG antibodies by ELISA. ***Results:*** All stool samples were negative for *Taenia* parasite eggs by microscopy. However, 42 individuals (4.2%) tested positive for *T. solium* taeniasis-specific IgG antibodies. Though statistically not significant, the IgG seropositivity was higher among individuals with lower education levels, low-income background, and higher frequency of hand washing. ***Conclusions:*** This is the first report from Kuwait and the Middle East on the detection of anti-*T. solium* taeniasis-specific serum IgG antibodies among the high-risk expatriate population. The results emphasize the importance of efficient and sensitive screening of *T. solium* carriers and prevention of infection transmission and development of cysticercosis in the local population.

B250

Study of Molecular profiling, Antimicrobial Susceptibility, and Virulence Factors of Mastitis-Causing *Staphylococcus chromogenes* in Nakhon Ratchasima, Thailand

Nanthanat Pansuksan, Mantana Jamklang ORCID iD, Wissarut Srisakvarangkool ORCID iD, Ekkasit Kanklang, Watsana Penkhrue ORCID iD, Chompunoot Wangboon

School of Preclinical Sciences, Institute of Science, Nakhon Ratchasima, Thailand

Abstract

Mastitis is a major problem for farmers and dairy farms due to the excessive use of antibiotics for treatment, which often leads to antimicrobial resistance and low milk quality. This study aims to investigate the staphylococcal mastitis in dairy farms in Pak Chong, Nakhon Ratchasima Province, Thailand. The methodology involved bacterial culture from cattle-mastitis samples and identifying species using MALDI-TOF MS. Antimicrobial susceptibility testing was done by disk diffusion method. Molecular profiling using Multi-Locus Sequence Typing (MLST) was performed. Detection of virulence factor genes of bacterial pathogens was conducted by PCR. Our results showed that of the 97 isolates of *Staphylococcus* species, 44 (45.36%) were *Staphylococcus chromogenes*, pointing out that this bacterium is the primarily staphylococcal mastitis cause. MLST molecular profiling of the 44 isolates revealed 15 novel sequence types of *S. chromogenes*. Antimicrobial susceptibility tests showed that 100% of these isolates were susceptible to cefoperazone, cefoxitin, erythromycin, and ciprofloxacin, and 63.64%, 72.73%, 81.82%, and 97.73%, were susceptible to tetracycline, sulfonamides/trimethoprim, clindamycin, and gentamicin, respectively. No virulence factors related to leukocidin, biofilm formation, or capsular polysaccharide were observed, suggesting that this bacterium does not cause severe mastitis in dairy cows. Our collected MLST data suggests that molecular epidemiology can be used to detect and monitor the spread of bacterial infections in the region offering important new information for the next dairy farm epidemiology research. Due to their potential to impact milk yield and quality, this study will highlight the significance of paying attention to standards and appropriate farm management.

B251

Protective efficacy of novel verocytotoxigenic Escherichia coli (VTEC) antigens as potential vaccine candidates.

Rhys Bruce, Yueran Hou, Conor Quinn, Siobhán McClean

University College Dublin, Dublin, Ireland

Abstract

Verocytotoxigenic *Escherichia coli* (VTEC) are a group of zoonotic foodborne pathogenic *E. coli* strains that causes bloody diarrhoea and Haemolytic Uraemic Syndrome (HUS) in more severe cases where young children under the age of 5 are particularly susceptible. While antibiotic treatment is contraindicated due to many patients experiencing severe symptoms and complications, there is an unmet need for a vaccine to reduce mortality and reduce the greatest risk of kidney failure in children. VTEC colonises gastrointestinal epithelial cells, therefore bacterial proteins involved in host-cell attachment represent promising vaccine candidates. Using a proteomic approach, we identified 14 novel proteins involved in the attachment of VTEC to two independent human gastrointestinal epithelial cell lines, HT29 or Caco-2. We previously showed that the glutamine-binding periplasmic protein, GlnH, had potential as a vaccine candidate, reducing bacterial colonisation in the colon and caecum by 1.25-log CFU.

Six additional proteins: Antigens A, P, G, D, M and L were selected for investigation as potential vaccine candidates. These were successfully cloned, expressed, and purified prior to immunisation in mice with the T-cell inducing, SAS adjuvant. Antigens A, P and G were highly immunogenic, stimulating a mixed Th1/Th2 response in immunised mice. Antigen A showed the greatest level of protection and reduced bacterial colonisation in the colon and caecum by 1.3-log CFU relative to vehicle only control ($p=0.0252$). It is evident that future VTEC vaccine developments should consider the combined use of adjuvants with antigens to provide greater protection against such an infection.

B252

Investigating the interplay between NKG2D ligands, SARS-CoV-2 infected epithelial cells and gamma delta T cells

Jamie James[1,2], Mark Bodman-Smith[1], Elisabetta Groppelli[1], Sally Sharpe[2], Javier Salguero-Bodes[2]

[1]City St. George's University of London, London, United Kingdom. [2]UK Health Security Agency, London, United Kingdom

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the aetiological agent of the coronavirus-19 disease (COVID-19) pandemic. Despite the efficacy of spike glycoprotein-targeting vaccines against SARS-CoV-2, the emergence of variants of concern (VOCs) that evade vaccine and natural immunity have been a continuous threat to public health. There is therefore a need to research and elucidate other immune responses to SARS-CoV-2 that are not affected by immune-evasion mutations. Gamma delta T cells (gd T cells) are an unconventional T cell that have both innate and adaptive immune response qualities and are understudied in the context of SARS-CoV-2 infection. In the case of influenza A virus (IAV) and SARS-CoV, gd T cells have been shown to respond to these viral infections by, for example, killing infected cells in a CD107a-dependent manner. Specifically, in IAV danger signals such as natural killer group 2 D (NKG2D) ligands are upregulated and activate gd T cells via NKG2D. We therefore postulate that gd T cells respond to SARS-CoV-2 infected cells in a similar manner. By using flow cytometry, we have investigated the expression profiles of two NKG2D ligands, MICA/B in SARS-CoV-2 infected cells of lung epithelial origin. Our data show that MICA/B are upregulated specifically in cells with active viral replication. Now, using a co-culture assay, we are investigating if gd T cells detect MICA/B upregulation on SARS-CoV-2 infected cells and respond to these cells, for example, by using CD107a as a surrogate marker for cytotoxicity.

B253

Determining the mechanism of killing of lectin-like protein antibiotics

Natasha Frate, Leighton Pritchard, Daniel Walker

Univerity of Strathclyde, Glasgow, United Kingdom

Abstract

In recent years there has been a global increase of antibiotic resistant infections. *Pseudomonas aeruginosa* is an important cause of hospital acquired infections that are associated with high levels of mortality. *P. aeruginosa* infections are becoming increasingly difficult to treat, with between 18 and 25% of clinical isolates showing multidrug resistance and isolated found across the globe which are resistant to all or nearly all available antibiotics.

Bacteriocins, antimicrobial proteins deployed by bacteria for competition and colonisation, offer a potential tool to combat antibiotic resistance. The narrow spectrum of bacteriocins, which generally target bacteria closely related to the producing strain, could enable targeting of specific pathogenic bacteria without causing damage to the wider microbiome. There has been a range of bacteriocins discovered against *P. aeruginosa,* known as pyocins, with lectin-like pyocin L1 showing efficacy in infection models. However, we currently lack understanding of the mechanism of action of pyocin L1, which limits its development as a potential antibiotic therapy.

Our current aim is to identify the molecular target of pyocin L1 in *P. aeruginosa* using a range of genomic, biochemical and biophysical analyses, We are also further testing the activity of pyocin L1 against *P aeruginosa* biofilms and determining its ability to enhance the activity of other anti-pseudomonal antibiotics.

B254

Screening of essential oils from the Lamiaceae family to produce a prototype, antimicrobial wound topical.

Narjis Aftab, Christopher Wallis, Sarah Hooper

Cardiff Metropolitan University, Cardiff, United Kingdom

Abstract

Chronic wounds are described as a silent epidemic, but the 2.8 million cases in the UK alone is on a par with cardiovascular disease and diabetes. Many topical, antimicrobial wound treatments exist but despite this, chronic wounds persist and do not heal. This project explored the use of essential oils from the Lamiaceae family, for which there is anecdotal evidence for successful use in wound healing, and investigated whether the most potent oils could be incorporated into to a gel for topical wound application.

Using Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), biofilm disruption and exclusion assays, the efficacy of thyme oil, basil oil, oregano oil, and sage oil were tested against the wound pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Of these, thyme oil had the best activity and was bactericidal to both planktonic and biofilm bacteria. The capacity to deliver this antimicrobial oil topically was investigated by incorporating it into a natural hydrogel (alginate) and a synthetic hydrogel (laponite). The addition of thyme oil to alginate destabilited it rendering it useless for topical application. The thyme oil-laponite gel was stable. Using agar diffusion assay and a dynamic chronic wound biofilm model, the thyme oil-laponite gel was observed to have excellent antimicrobial activity, resulting in 2-3 log reductions in bacteria.

In conclusion, thyme oil has potential to be developed further as a topical wound treatment, however, it will be vital to assess the toxicity of thyme oil to human skin cells in a suitable *in vitro* model.

B255

Identifying genetic determinants in *Pseudomonas aeruginosa* contributing to phage resistance.

Rama Bhatia ORCID iD, Anna Richmond, Stineke van Houte

University of Exeter, Cornwall, United Kingdom

Abstract

P. aeruginosa is a multidrug resistant opportunistic pathogen which presents a major challenge in clinical settings due to its ability to rapidly acquire resistance mechanisms. Bacteriophages (phages) are being increasingly explored as alternative therapeutic agents against antibiotic-resistant bacteria. Understanding the genetic factors underlying phage resistance can facilitate the development of more effective phage therapies.

This study focuses on profiling phage infection in clinical isolates of *P. aeruginosa*. We combine high-throughput phenotypic assays with genomics to assess phage susceptibility and resistance in a large and diverse collection of isolates. The resulting profiles reveal a spectrum of patterns, categorized into three phenotypic levels: susceptible, intermediate resistance, and complete resistance. We aim to integrate genome-wide association studies (GWAS) with pan-genome analysis to identify specific genetic determinants associated with resistance to individual phages or broader resistance patterns.

Our findings have the potential to offer insights into the diversity of phage resistance mechanisms in *P. aeruginosa* and highlight genetic determinants that could inform targeted phage therapy strategies. By advancing our understanding of phage-host dynamics in clinical pathogens, this research contributes to the growing body of knowledge on utilizing phages to combat antibiotic-resistant infections.

B256

Evolutionary adaptation of Burkholderia multivorans to the novel antibiotic enacyloxin IIa leads to phenotypic and genetic changes

Lucile Hubert ORCID iD, Cedric Berger, Eshwar Mahenthiralingam

Cardiff University, Cardiff, United Kingdom

Abstract

Introduction

Burkholderia multivorans is problematic multidrug-resistant (MDR) pathogen infecting people with cystic fibrosis. It often requires a combination of antibiotics to treat these infections. Novel therapeutics are needed to control infections by MDR organisms. Enacyloxin IIa is a polyene antibiotic produced by *Burkholderia ambifaria* and *Burkholderia gladioli* and was investigated as a potential therapeutic against *B. multivorans*.

Materials and methods

An evolutionary adaptation experiment was performed on *B. multivorans* by repeatedly exposing the bacterium to increasing doses of enacyloxin IIa. The resulting isolate (*B. multivorans* EnaTr) was then tested for alterations in antibiotic susceptibility and other phenotypic properties. Whole-genome (WGS) and RNA sequencing were performed on EnaTr to understand the molecular responses to enacyloxin IIa exposure.

Results

The minimum inhibitory concentration of *B. multivorans* EnaTr to enacyloxin IIa increased from 4 µg/mL to 24 µg/mL in fourteen days. Susceptibility to therapeutic antibiotics for *Burkholderia* infections decreased in EnaTr. Microbroth dilution assays showed a significant increase in susceptibility to aminoglycosides for the EnaTr derivative. Use of biomimetic medium, SCFM2, revealed increased susceptibility to most antibiotics for *B. multivorans* except trimethoprim compared to growth in standard medium. WGS identified only 3 single-nucleotide polymorphisms in EnaTr and gene expression analysis highlighted an increased expression of ribosomal proteins.

Conclusion

Repeated exposure to enacyloxin IIa increases the tolerance of *B. multivorans* to this novel therapeutic and to other antibiotics. However, adaptation to enacyloxin IIa was accompanied by increased susceptibility to aminoglycosides, leaving a potential therapeutical route despite the resistance to enacyloxin IIa.

B257

Microbial colonisation of bed linen and its importance in establishing bed changing policies in healthcare and hospitality

Caroline Cayrou, Marina Abu-Aita, Katie Kaird

De Montfort University, Leicester, United Kingdom

Abstract

Background. There is no bed changing policy for long stays in healthcare and hospitality settings. Little is known about the dynamic microbial colonisation of bed linen and its role in infection. Studies exploring microbial colonisation of bed linen mainly focus on bacterial species and only assess one time-point.

Aim. This study aimed to explore variation in microbial concentration and diversity over 1 month of usage of bed linen.

Methods. Nine volunteers used polycotton bed linen for 3 days, 1, 2, 3 and 4 weeks. After use, 25cm2 swatches were cut from the textile at the hips, feet and head resting sites. Organisms were recovered from the swatches and their concentration was estimated using Standard Plate Count Agar and Sabouraud plates incubated aerobically at 37°C and 30°C respectively. The samples were then assessed by 16S metagenomic analysis.

Results. On average, 3.9 $\log_{10}$CFU/25cm^2 (bacteria) and 3.6 $\log_{10}$CFU/25cm^2 (fungi/yeast) could be recovered from the bed linen swatches. No significant increase of microbial concentration was observed with longer usage time, there was a trend of increasing microbial load in the feet area over 2 weeks. Initial metagenomic analysis demonstrated the presence of skin microorganisms.

Conclusions. There was no additional build-up in concentration of microorganisms after 3 days suggesting a turnover in presence of microorganisms was occurring. This was further explored in the change of diversity of species over time. Better understanding of bed linen colonisation will allow for informed bed linen policies to be developed for number of public areas.

B258

Up-regulation of the protease aureolysin contributes to dairy niche adaptation by *Staphylococcus aureus*

Amy Pickering ORCID iD[1], Jamie Gorzynski[1], Grace Taylor-Joyce[2], Willow Fox[1], Pedro Melo[1], Joana Alves[1], J. Ross Fitzgerald[1]

[1]Roslin Institute, Edinburgh, United Kingdom. [2]University of Edinburgh, Edinburgh, United Kingdom

Abstract

Staphylococcus aureus is a multi-host pathogen and a leading cause of bovine mastitis, a disease with major welfare and economic impacts to the global dairy industry. We have discovered that when cultured in bovine milk, most *S. aureus* isolates from bovine-associated lineages mediate clotting, produce robust milk-associated biofilms, and exhibit enhanced growth compared to *S. aureus* isolates from human infection. Using molecular microbiology and functional analysis, we have discovered that upregulation of the extracellular protease aureolysin is required for milk clotting by bovine *S. aureus.* Through casein-digestion, the major protein constituent of milk, this aureolysin-mediated milk clotting leads to enhanced growth and biofilm formation in milk. In contrast, the overexpression of aureolysin in human-adapted strains is sufficient to promote milk clotting but does not confer an enhanced growth phenotype indicating that additional metabolic adaptations are required for enhanced growth. Comparative genomic analysis of genetically related pairs of *S. aureus* isolates with distinct milk clotting phenotypes, revealed different mutations in two-component systems in different lineages, that correlated with enhanced aureolysin expression and milk clotting. Taken together, we report that the convergent upregulation of aureolysin expression in different *S. aureus* lineages is a key niche-adaptive trait of bovine *S. aureus*.

B259

Advancing Sepsis Treatment: Synthesis and Evaluation of Multi-Target Drugs with Antimicrobial, Antioxidant and Anticoagulant Properties.

Jordan West, Paul Kong Thoo Lin, Aakash Welgamage Don, Graeme Kay, Gael Morrow

Robert Gordon University, Aberdeen, United Kingdom

Abstract

Sepsis is a complex disease triggered by infection, causing hyperinflammation, oxidative stress, excessive blood coagulation and death in severe cases. The complex nature of the sepsis makes single-target drugs ineffective in most cases, resulting in approximately 11 million deaths worldwide each year. This study aims to addresses these challenges by synthesising and evaluating a library of eleven Naphthalimide-based multi-target drugs (MTDs) designed to act on multiple mechanisms involved in sepsis.

Compounds JW1-11 were characterised using high-resolution-mass spectrometry and nuclear magnetic resonance. Antimicrobial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus* through minimum inhibitory concentration (MIC) and time-kill kinetics assays. Lead compounds, JW6 and JW10 demonstrated MIC values of 125 µg/mL against *E. coli* and 250 µg/mL against *S. aureus*. The antioxidant potential of the compounds was assessed using DPPH and FRAP assays, showing IC_{50} values for DPPH 40.70 µM and 38.90 µM (JW6 and JW10), with JW10 showing good antioxidant activity in the FRAP assay (TE = 1.25). The clot lysis assay was conducted to investigate the compounds' anticoagulant properties. Compounds JW1, 4, 9 and 11 exhibited strong fibrinolytic activity, with the lowest 50% clot lysis time of 68-78 minutes, compared to the control (70 min). Compounds JW3, 5, 7 and 8 showed the lowest maximum absorbance of 0.2, compared to the control (0.4 absorbance at 405nm). With the potential to simultaneously addressing the infection, oxidative stress and blood clotting of sepsis, the compounds, particularly JW10, show promise and provide ideal candidates for further evaluation.

B260

Unveiling the molecular basis of invasive Non-Typhoidal Salmonella within human macrophages by dual-RNAseq

Natalia Cattelan ORCID iD, Francesco Amadeo, Lijuan Luo, Jay Hinton

University of Liverpool, Liverpool, United Kingdom

Abstract

Non-typhoidal *Salmonella* (NTS) are a group of bacteria that cause gastroenteritis in humans. Certain serovars, particularly *Salmonella* Typhimurium and *Salmonella* Enteritidis, have developed the ability to spread from the gut, enter the bloodstream, and cause a systemic or invasive disease. This has recently become a significant health concern in Sub-Saharan Africa, disproportionally affecting individuals with weakened immune system, resulting in an annual mortality rate of around 66,500.

Little is known about the infection biology of invasive non-typhoidal Salmonella (iNTS) pathogens. To establish a systemic infection, these iNTS must be able to infect, survive and eventually replicate within host cells, including epithelial cells, dendritic cells and macrophages. Although macrophages are at the front line of host defence against bacterial pathogens, they are a crucial colonization niche for *Salmonella.* Replication within macrophages allows Salmonella to establish a systemic disease in susceptible hosts. We investigated the ability of *S.* Typhimurium and *S.* Enteritidis isolates from iNTS patients to invade and survive within human macrophages. We observed an increased replication in both *S.* Typhimurium and *S.* Enteritidis iNTS compared to gastroenteritis isolates. We analysed cytotoxicity levels in these infected cells and NO and ROS production, without finding any differences between isolates. With a dual-RNAseq approach, we looked into the transcriptome of *Salmonella* isolates simultaneously with the host transcriptome to unveil differences between invasive and non-invasive *Salmonella*, and to understand the host response to these infections.

B261

Where do pathogens live? Mapping the microbial hiding spots of pathogenic *Streptococcus suis* in pigs and farms.

Chenxi Liu[1], Bart Vanderhee ORCID iD[2], Lucy Steele[1], Rachel Hewitt ORCID iD[1], Jerry Wells[2], Dan Tucker[1], Lucy Weinert[1]

[1]University of Cambridge, Cambridge, United Kingdom. [2]Wageningen University & Research, Wageningen, Netherlands

Abstract

Streptococcus suis, a common member of the pig respiratory microbiota, is a zoonotic pathogen responsible for pneumonia and invasive disease in pigs and humans. Despite interventions that reduce morbidity, *S. suis* continues to impose significant economic burdens on the swine industry, with infected pigs showing slower growth and incurring higher veterinary costs.

Pathogenic *S. suis* lineages, primarily associated with severe disease and zoonotic infections, emerge from a diverse commensal population in the upper respiratory tract. These pathogenic strains are defined by three genomic islands linked to host-pathogen interactions. This leads us to hypothesise that commensal and pathogenic *S. suis* occupy distinct ecological niches within the host and its environment.

To investigate this hypothesis, we used fluorescence *in situ* hybridization, confocal microscopy, and quantitative PCR to map the distribution of commensal and pathogenic *S. suis* in pig hosts and the farm environment. Our findings indicate that while both ecotypes are prevalent in the upper respiratory tract, the oral cavity has the greatest abundance of *S. suis* DNA. Pathogenic *S. suis* on the other hand, was more often found in the nasal cavity. Furthermore, we detected *S. suis* DNA in feeders, suggesting that cross-contamination between pig saliva and feeding equipment may contribute to within-farm spread.

In conclusion, this study identifies the nasal cavity as a key reservoir for pathogenic *S. suis* and demonstrates how genomic and ecological dynamics drive bacterial pathogenicity. Our findings have implications for designing targeted strategies to manage pathogenic strains in swine populations.

B262

Bacteria of a Feather Cluster Together: A Machine Learning AI view of strain differences

Lisa Crossman ORCID iD

Norwich, Norwich, United Kingdom

Abstract

Whilst the study of comparative genomics and phylogenetics have aided epidemiology and diagnostics for a few years, distinguishing pathogenic bacterial strains have traditionally focussed on single nucleotide differences (SNPs), multi-locus sequence typing or the presence and absence of specific genes across pangenomes.

However, understanding the barriers to genetic exchange between distinct phylogenetic clades is also important. What genetic mechanisms render one clade separate from another? Potential barriers to genetic exchange may arise from separated ecological niches, geographical or temporal isolation, and factors that alter or limit horizontal gene flow. Such factors include environmental stressors, restriction-modification systems, CRISPR immunity, differences in %GC content and codon usage, plasmid incompatibility and maintenance, and mechanisms affecting DNA acquisition by transformation (the uptake of DNA from the environment), transduction (introduction of DNA *via* bacteriophages) and conjugation (transfer of DNA mediated by pili structures).

Here we introduce a machine learning explainable AI workflow that is simple to use, with the ability to identify key differences between strains from distinct clades. Examples are drawn from datasets of coagulase negative staphylococci and *Campylobacter spp*. This approach offers fresh insight into the genetic factors that contribute to clade separation, advancing our ability to analyse pathogenic strains.

B263

Flagellar differences may contribute to the host specificity of *Salmonella* serovars

Annis Newman[1], Jochen Arlt[1], Aidan Brown[1], Mark P Stevens[2], Prerna Vohra[1]

[1]The University of Edinburgh, Edinburgh, United Kingdom. [2]The Roslin Institute, Edinburgh, United Kingdom

Abstract

Non-typhoidal *Salmonella* is a major global cause of human infections commonly acquired via the food chain from farmed animals. Generalist *Salmonella* serovars cause gastroenteritis in a broad range of hosts (e.g., *S.* Typhimurium in humans, cattle and pigs) while host-restricted serovars cause typhoidal disease that can lead to asymptomatic gallbladder carriage once infection resolves (e.g., *S.* Dublin in cattle, *S.* Choleraesuis in pigs). The mechanisms underlying this differential virulence and host specificity of serovars are currently ill-defined. During infection *Salmonella* encounters bile in the intestines and the gallbladder at low and high concentrations, respectively. Here, we used RNAseq to study serovar responses to bile at different concentrations and from different hosts to determine if bile responses are associated with host specificity. We found that *S*. Typhimurium consistently expressed flagellar genes at higher levels compared to *S*. Dublin and *S*. Choleraesuis when cultured in lysogeny broth (LB) or bile, despite a general reduction in gene expression across serovars in the presence of bile. *S*. Typhimurium also showed increased swimming and swarming motility compared to *S*. Dublin and *S.* Choleraesuis in LB and bile. Further, using differential dynamic microscopy, we observed a greater proportion of motile cells in *S*. Typhimurium cultures compared to *S*. Dublin and *S.* Choleraesuis. These observations indicate that host-restricted serovars reduce the expression of flagellin, an agonist of innate immunity, to evade detection in ways that may lead to systemic spread, and that bile suppresses flagellin expression in all the above mentioned non-typhoidal serovars irrespective of their host-specificity.

B264

Rapid detection of Mycobacterium tuberculosis with novel solvatochromic fluorophore probes

Sonali Chhabra, Raman Parkesh

CSIR-Institute of Microbial Technology, Chandigarh, India

Abstract

Tuberculosis (TB) remains a concerning global health challenge, and therefore, there is an urgent need for point-of-care tuberculosis diagnostic methods that are rapid, inexpensive, and easy to use in clinical settings. Here we report a solvatochromic dye-benzothiazinone (BTZ) conjugate for rapidly detecting *Mycobacterium tuberculosis* (Mtb) in minutes. The fluorophore probe comprises a benzo[de]isoquinoline-1,3-dione dye, a linker, and a BTZ-based targeting group for anchorage to mycobacterial cell wall enzyme DprE1. The conjugate exhibits solvatochromic behavior with respect to the environment. The dye produces a detectable fluorescent signal once it is incorporated into the bacterial cell wall because of the change in the local environment. The fluorophore probe inhibits the DprE1 enzyme function and exhibits anti-tubercular potency against *Mycobacterium tuberculosis*. Remarkably, we detected fluorescent mycobacterium cells within 10 minutes of incubation with the probe. Thus, the probe allows rapid visualization of Mtb that has the potential for enhanced Mtb detection at the point-of-care in resource-limited environments.

B265

Genomic and phenotypic changes associated with the acquisition of fluoroquinolone resistance in *Campylobacter jejuni*

Shafik Oubihi, Marguerite Clyne, Tadhg Ó Cróinín

University College Dublin, Dublin, Ireland

Abstract

Campylobacter jejuni is the leading cause of bacterial gastroenteritis worldwide, with fluoroquinolone resistance posing a persistent challenge despite regulatory interventions. Resistance in *C. jejuni* is associated with mutations in the *gyrA* gene which lead to a relaxation of DNA supercoiling. This is known to affect several key phenotypes, including motility, biofilm formation, and virulence, with important implications for transmission and severity of disease. In this study, two laboratory and two recently isolated *C. jejuni* strains were repeatedly passaged on increasing concentrations of ciprofloxacin, yielding isolates with low (up to 10µg/mL), medium (up to 40µg/mL), and high levels (>40µg/mL) of ciprofloxacin resistance. PCR confirmed the presence of the T86I mutation in the *gyrA* gene of these resistant isolates, and motility testing via the soft agar method revealed strain-dependent differences between susceptible and resistant isolates. Fluorescence microscopy was used to assess viability and structure of biofilms under microaerobic and aerobic conditions, with changes in biofilm morphology observed in resistant isolates. Additionally, assays were conducted to evaluate changes in protein secretion and aerotolerance, and chloroquine gel electrophoresis was used to compare plasmid supercoiling levels between susceptible and resistant isolates. Isolates were whole genome sequenced to identify shared SNPs associated with the phenotypic shift that accompanies fluoroquinolone resistance. Results to date build on previous observations demonstrating that the acquisition of resistance-conferring mutations in *gyrA* leads to changes in phenotypes with implications for *C. jejuni* transmission and virulence.

B266

Lactobacilli isolated from the chicken caecum with antimicrobial activity are probiotic candidates for controlling Salmonellosis in poultry

Schwann Chuwatthanakhajorn ORCID iD[1,2], Witthawat Wiriyarat ORCID iD[2], Amy Wedley ORCID iD[1], Amy Watts ORCID iD[1], Paul Wigley ORCID iD[1,3], Rachael Slater ORCID iD[4], Kraig Green ORCID iD[4], Chris Probert ORCID iD[4], Barry Campbell ORCID iD[1]

[1]Infection Biology & Microbiomes, University of Liverpool, Liverpool, United Kingdom. [2]Faculty of Veterinary Science, Mahidol University, Salaya, Thailand. [3]Bristol Veterinary School, Infection & Immunity, University of Bristol,, Bristol, United Kingdom. [4]Molecular & Clinical Cancer Medicine, University of Liverpool, Liverpool, United Kingdom

Abstract

With the trend shifting towards reducing antibiotic usage, the utilization of Lactobacilli as a probiotic is emerging as an alternate, effective method for mitigating *Salmonella* infection in poultry . Here, we investigated the antimicrobial activity of cell-free culture supernatants (CFCS) of three Lactobacilli isolated from chicken caecum, including *L. johnsonii*, *L. reuteri* and *L. crispatus*, against five *Salmonella enterica* serovars (*S.* Typhimurium 4/74, *S.* Enteritidis P125, *S.* Enteritidis AviPro®, *S.* Gallinarum 9 and *S.* Gallinarum 287/91). CFCS from each *Lactobacillus* strain showed potent antibacterial activity against all *Salmonella* serovars. The degree of inhibition varied between *Lactobacillus* strains and their action on *Salmonella* serovars. The minimum inhibitory concentration (MIC) of *Lactobacillus* CFCS against *Salmonella* ranged from 9.33±0.61 to 21.55±7.49 mg/mL, and minimum bactericidal activity (MBC) ranged from 12.62±2.10 to 25.54±9.88 mg/mL. In agar diffusion assays, *L. crispatus* demonstrated the highest inhibitory effect, against *S.* Typhimurium 4/74, *S.* Enteritidis P125 and *S.* Enteritidis AviPro®. *S.* Gallinarum showed highest susceptibility amongst the serovars to Lactobacilli CFCS. *Lactobacillus* CFCS also demonstrated ability to suppress *Salmonella* invasion of 8E11 chicken caecal enterocytes compared to untreated controls. Size fractionation indicated that the antimicrobial metabolites of CFCS are £3kDa, resistant to proteases and active at pH >2 but <7. Analysis by head-space solid-phase microextraction (HS-SPME)-gas chromatography/mass spectrometry (GC/MS) identified short and medium-chain fatty acids as anti-*Salmonella* metabolites generated by *Lactobacillus*. In conclusion, *L. johnsonii*, *L. reuteri* and *L. crispatus* have potential as probiotic candidates for controlling salmonellosis in poultry.

B267

Exploring host ligand binding capabilities of Opacity proteins in *Neisseria gonorrhoeae*

Elliot Macdonald[1], Werner Pansegrau[2], Silvana Savino[2], Darryl Hill[1]

[1]University of Bristol, Bristol, United Kingdom. [2]GSK vaccines, Siena, Italy

Abstract

Opacity proteins (Opa) are a family of hypervariable outer membrane proteins in *Neisseria gonorrhoeae* that function as bacterial adhesins. The majority of characterised Opa proteins bind the human receptor family Carcinoembryonic antigen-related cell adhesion molecules (CEACAM), whilst one member, Opa50, binds the regulatory glycoprotein vitronectin. Binding of the latter requires polysaccharide bridging molecules, such as glycosaminoglycans, and increases adhesion and invasion of host cells. Using a panel of isogenic mutants expressing one defined Opa, purified vitronectin and CEACAM1 binding was assessed using techniques including ELISA and co-sedimentation assays. Expression of each Opa examined increased vitronectin binding, indicating previously unappreciated Opa-vitronectin interactions. Across isolates binding was lost in the presence of NaCl, demonstrating the importance of ionic forces in these interactions. Growth on agarose-based media, rather than agar, abrogated vitronectin binding, highlighting that agar-acquired polysaccharides can also act as bridging molecules for multiple Opa in the Opa-polysaccharide-vitronectin axis. Contrastingly, CEACAM1 binding was increased for certain Opa-expressing isolates when grown on agarose-based media, suggesting agar-acquired polysaccharides play an inhibitory role in the direct Opa-CEACAM binding. Further investigation of the interplay between Opa ligands found that glycosaminoglycan polysaccharides can impact CEACAM binding to Opa-expressing gonococcal isolates, though vitronectin was found to have no influence. Together, this work expands those Opa proteins known to interact with vitronectin, which facilitates increased gonococcal invasion into host cells. The mechanism of binding appears to be common amongst Opa proteins, constituting an ionic Opa-polysaccharide-vitronectin binding axis.

B268

Development of Rapid, High-Resolution Diagnostic Models for Field Deployment: A Cholera Case Study

Vignesh Shetty ORCID iD[1], Fahad Khokhar[2], Goutam Chowdhury[3], Ayyanraj Neeravi[4], Balaji Veeraraghavan[4], Debasish Samal[5], Jyotirmayee Turuk[5], Bibhuti Bhusan Pal[5], Sunitha Pakalapaty[6], Nagamani K[6], Rebecca Stout[7], Mamatha Ballal[8], Sabu Thomas[9], Asish K Mukhopadhyay[3], Bhabatosh Das[10], Thandavarayan Ramamurthy[3], Ankur Mutreja[2], Nicholas R Thomson[1]

[1]Wellcome Sanger Institute, Cambridge, United Kingdom. [2]University of Cambridge, Cambridge, United Kingdom. [3]National Institute for Research in Bacterial Infections, Kolkata, India. [4]Christian Medical College, Vellore, India. [5]Regional Medical Research Centre, Bhubaneswar, India. [6]Gandhi Medical College and Hospital, Secunderabad, India. [7]Imperial College London, London, United Kingdom. [8]Kasturba Medical College, Manipal, India. [9]Rajiv Gandhi Center for Biotechnology, Trivandrum, India. [10]Translational Health Science and Technology Institute, Faridabad, India

Abstract

Cholera, caused by *Vibrio cholerae*, remains a major public health threat, particularly in regions with inadequate sanitation. Rapid and accurate detection is crucial for effective outbreak management, but current diagnostic methods often face delays due to time-intensive procedures and the need for confirmatory testing. While several cholera rapid diagnostic tests (RDTs) are available, most are for research use only (RUO) and none are prequalified by WHO, highlighting a critical gap in diagnostics. To address this, we developed a PCR-oligo chromatographic assay (PCR-OC) and a qPCR model for direct detection of *V. cholerae* in stool samples, designed as Rapid Diagnostic Confirmatory Tests (RDCTs). These assays specifically target pandemic and epidemic strains, including 7PET, cholera toxin, and O1/O139 serogroups. Both assays are compact and portable, fitting within a "lab in a bag" model ideal for field use. The PCR-OC provides results in under two hours, while the qPCR model delivers results in under one hour. Analytical validation of PCR-OC demonstrated high specificity with a detection limit of 4.3 ng/µl gDNA (qPCR validation is ongoing). Clinical validation on 534 stool samples across seven sites in India showed 100% sensitivity, 83% specificity, and 85% accuracy, with a successful field trial in Malawi confirming its utility during outbreaks. The qPCR model, pending full clinical validation, is more affordable (<£4 per test) than PCR-OC (<£7). These assays offer a rapid, cost-effective solution for high-resolution cholera detection, supporting WHO and GTFCC goals for cholera surveillance and outbreak response.

B269

Development of a Multi-Species Wound Biofilm Model, and Potential Diagnostic Applications

Immaculata Ede ORCID iD, Darren Reynolds, Robin Thorn

University of the West of England, Bristol, United Kingdom

Abstract

There is a need to model wound biofilms *in vitro* to enable research and development of novel diagnostic and therapeutic approaches. Traditionally, these *in vitro* studies of clinically relevant biofilms have involved their growth on solid (e.g. plastic) surfaces, and do not adequately simulate the wound environment *in vivo*.

The main aim of this study was to develop a collagen based multispecies wound biofilm model that is more representative of a wound environment *in vivo* and to exploit this model to investigate microbial volatile compounds as a potential diagnostic tool.

Four species of bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*) that are frequently present in clinically relevant polymicrobial wound infections were cultured on a semi-solid collagen gel matrix, housed within a drip flow reactor and constantly perfused with simulated wound fluid. Mean biofilm densities of 10.31, 10.04, 11.20 and 10.39 CFU Slide^{-1} for *S. aureus*, *E. coli*, *P. aeruginosa* and *S. pyogenes* respectively were achieved after 48 hours of co-culture, whereby mature biofilms were visualised using Scanning Electron Microscopy.

Using Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) coupled with multivariate statistical analysis, the four species could be differentiated based on detection of volatile compounds when grown as monospecies biofilms. Work is ongoing to determine if the same volatile compounds can distinguish them when grown within a polymicrobial community.

We have successfully developed a multispecies wound biofilm model and demonstrated its use in microbial volatile compound investigation.

B270

Prevalence of Sexually Transmitted Infections (STIs) in Pregnant Women and Breastfeeding Mothers in some Rural Communities in Abia State, Nigeria

Amaka Awanye ORCID iD, Chidozie Ibezim, Catherine Stanley

University of Port Harcourt, Port Harcourt, Nigeria

Abstract

Sexually transmitted infections (STIs) are common infections in Nigeria, especially among women of childbearing age. Untreated infections in pregnancy and breastfeeding also pose a huge risk to the foetus and infant. The objectives of the study were to determine the prevalence of some STIs and their associated risk factors among pregnant and nursing mothers in some rural communities in Abia State, Nigeria. Socio-demographic parameters were obtained from 110 consenting participants via pre-tested structured questionnaires. Blood, urine and high vaginal swab samples were used to test for STIs using relevant microbiological and immunological laboratory tests. The results obtained from 43 pregnant women and 67 breastfeeding mothers revealed the presence of gonorrhoea (6.4%), trichomoniasis (10.9%), HIV (1.8%) and HBV (3.6%). Syphilis and HCV were not detected. Associated risk factors identified include a history of STI, urinary tract infection, miscarriage, stillbirth, employment status and level of education. Infection was most prevalent among unemployed married women aged between 18 and 29 years, with secondary school education as their highest level of education. The mean parity of women with infection was 2.42 ±0.29. Our findings revealed a low prevalence of STIs among pregnant and nursing mothers despite low levels of education and lack of employment among the infected participants. Public enlightenment, awareness and screening for prophylactic treatment are still encouraged to reduce the burden of STIs among vulnerable populations.

B271

Infection studies reveal high lethality of methicillin susceptible *Staphylococcus aureus* ST398 in animal models

Miquel Sánchez-Osuna[1,2], Marc Bravo[2,3], María A. Cañas[4], Andrómeda-Celeste Gómez[2,3], Inmaculada Gómez-Sánchez[1,2], Paula Bierge[1,2], Marc Pedrosa[1], Mateu Espasa[4], José M. Miró[4], Isidre Gibert[2,3], Oriol Gasch[1], Cristina García-de-la-Mària[4], Daniel Yero[2,3], Oscar Q. Pich[1,2]

[1]Hospital Universitari Parc Taulí, Institut d'Investigació i Innovació Parc Taulí, Sabadell, Spain. [2]Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain. [3]Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain. [4]Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain

Abstract

To identify genetic factors of *Staphylococcus aureus* associated with mortality, we evaluated the lethality in *Galleria mellonella* larvae of a collection of 77 *S. aureus* strains from dominant lineages isolated from patients with bacteremia. Infection assays revealed heterogeneous LT50 (Lethal Time 50) across the different lineages. Strains from the ST398 lineage showed a hypervirulent phenotype (LT50 = 24.8h ± 8.7 SD), while isolates from the CC30 lineage displayed low lethality (LT50 = 80.0h ± 18.4 SD). In vitro analyses revealed high α- and δ-hemolytic activity in ST398, while CC30 isolates exhibited low hemolytic activity. Of note, we identified an ST398 isolate with an anomalously low lethality (LT50= 48.5h). This isolate, which also showed low hemolytic activity in vitro, carried a transposon inactivating the Agr system; such insertion was not observed in any other strain of the ST398 lineage. The lethality of three ST398 isolates, including the one with the inactivated Agr, and two CC30 strains was assessed in an experimental rabbit model of infective endocarditis. The results obtained confirmed the high lethality of the ST398 lineage, revealing a remarkable correlation between the results in *G. mellonella* and the rabbit model. Transcriptome analyses revealed higher expression in ST398 strains of several key toxins (e.g., *hly/hla*, *hld*, *hlgB*) and virulence factors (e.g., *agrB*, *agrC*, *scn*, *hysA*, *geh*, *coa*, *ebp*) when compared to CC30 strains. Our study demonstrates that *G. mellonella* is an effective model for identifying hypervirulent *S. aureus* strains and facilitating the identification of bacterial genetic factors of mortality

B272

HSV-1 disrupts cellular tight junctions through viral:host protein interactions

Daniela Carter-Lopez, Henry Barrow, Vivienne Connor, Colin Crump, Stephen Graham

University of Cambridge, Cambridge, United Kingdom

Abstract

Herpes Simplex Virus (HSV)-1 is a widespread, double-stranded DNA virus that generally causes orofacial or genital lesions but can cause more severe outcomes like viral encephalitis. While acyclovir and valacyclovir are highly effective at treating symptoms of HSV-1 infection, they are not curative and HSV-1 persists in the host for life. The HSV-1 protein pUL21 has multiple roles during HSV-1 infection: it is important for virus assembly and cell-to-cell spread. pUL21 interacts with multiple cellular and viral partners. Notably, pUL21 binds the cellular phosphatase PP1 and recruits it to cellular or viral targets to mediate their dephosphorylation. Combining proximity labelling (BioID2) of pUL21 tagged at the endogenous locus with quantitative mass spectrometry, we have identified that pUL21 localises with multiple proteins that reside at cellular (tight) junctions. Additionally, fluorescence microscopy results an unexpected role for pUL21 in the disassembly of cell junctions during HSV-1 infection. We identify that pUL21 forms a weak-affinity interaction with a cellular tight junction adaptor protein, which becomes dispersed from the cell junctions during HSV-1 infection. This adaptor protein is a central scaffold that connects cell junctions to the actin cytoskeleton, and it also acts as a platform for numerous cell signalling pathways. Our data suggests that HSV-1 exploits an unanticipated mechanism to promote viral cell-to-cell spread.

B273

The Sticking Point of the Commensal to Pathogen Switch in *Staphylococcus aureus*: Comparing Biofilm Phenotype and Genotype between Infection and Nasal Strains from the Same Patients

Hugh White ORCID iD[1], Kasia Parfitt ORCID iD[2], Bernadette Young ORCID iD[3], Daniel Wilson ORCID iD[4], Justine Rudkin ORCID iD[5]

[1]Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom. [2]Department of Biology, University of Oxford, Oxford, United Kingdom. [3]Nuffield Department of Medicine, Oxford, United Kingdom. [4]Big Data Institute, University of Oxford, Oxford, United Kingdom. [5]School of Infection and Immunity, University of Glasgow, Glasgow, United Kingdom

Abstract

Staphylococcus aureus is a common commensal, but also a major pathogen. There is conflicting evidence regarding the propensity of different clones to become pathogenic. Part of this may be due to the diverse virulence mechanisms *S. aureus* possess. Determining which traits are essential for pathogenesis is key to determining the risk posed by different clones. One such virulence factor is biofilm, with the production of PIA-independent biofilm being associated with MRSA and PIA-dependent biofilm production being associated with MSSA.

We aimed to determine whether there were distinct genetic determinants of biofilm production between colonising and infection isolates. We conducted biofilm assays on 105 pairs of *S. aureus* strains (colonising and infection) isolated from the same patient in media supplemented with glucose and sodium chloride to induce production of PIA-independent and PIA-dependent biofilm types. This was followed up with nucleotide and protein kmer genome-wide association studies (GWAS) on the assembly contigs for each isolate.

Whilst overall, we could not find a significant pattern in biofilm production between infection and carriage isolates, there were clear genetic differences; after mapping to the *S. aureus* ED98 genome, significant associations were found between nucleotide variants in a gene encoding an LPXTG-motif cell wall anchored protein and PIA-independent biofilm; and between nucleotide variants in a putative phage-derived amidase and PIA-dependent biofilm production, previously found in a host jump event from humans to chickens. These potentially novel determinants of biofilm formation in *S. aureus* require further work to determine their exact role in biofilm and infection.

B274

Characterisation of novel bacteriophages therapeutic potential against cattle pathogen *Moraxella bovis*.

Hannah Sampson ORCID iD[1], Malgorzata Wegrzyn[1], Andrew Millard[1], Greg Firth[2], Rajesh Odedra[2], Martha Clokie[1]

[1]Leicester, Leicester, United Kingdom. [2]Carus Animal Health, Surrey, United Kingdom

Abstract

Infectious bovine keratoconjunctivitis (IBK) is the most important ocular disease of cattle worldwide and has a considerable negative impact on cattle welfare. It is a highly contagious disease, with outbreaks common in the warmer months. The infection is caused by *Moraxella bovis* and is characterised by four disease stages with varying degrees of corneal opacity, ulceration and, in severe cases, blindness. Attempts to vaccinate cattle against IBK, have been unsuccessful.

Currently, antibiotic medication is the predominant IBK treatment, and treatment requires expert cattle handling skills to administer. Furthermore, as antibiotic resistance is one of the biggest threats to global health and food security, developing a more targeted therapeutic approach is needed. One solution could be, to exploit naturally occurring bacteriophages, to treat this difficult and debilitating infectious disease. Potentially the phages would replicate at the sight of infection thus requiring less contact to remove disease symptoms.

To explore phage use in IBK, we isolated the first known *Moraxella bovis* phages and characterised them according to their genome sequence and with transmission electron microscopy. Their potential to be developed as therapeutics in terms of virulence and host range was then assessed using a library of clinically relevant and geographically diverse strains of *Moraxella bovis.*

B275

Phase variation of Moraxella catarrhalis adhesins: Impact on CEACAM binding and biofilm formation

Tom Gerrard ORCID iD

University of Bristol, Bristol, United Kingdom. University of the West of England, Bristol, United Kingdom

Abstract

Background:

Moraxella catarrhalis (Mx) is an opportunistic pathogen which colonises the human respiratory tract. Mx has the ability to cause a variety of infections and, with a growing number of infections and rapid spread of antibiotic resistance in Mx populations, it is vital to understand more about this underappreciated pathogen.

Methods:

In this study we selected a sub-population of Mx clinical isolates providing representative variation in isolation site, age of host and MLST type. Two phenotypes relevant to Mx pathogenesis are explored here, binding to CEACAM (Carcinoembryonic antigen-related cell adhesion molecules) measured by western blotting and ELISAs and biofilm formation measured by crystal violet staining.

Results:

Here, we demonstrate that strains expressing the variant UspA2 adhesin (UspA2V) which belong to ribotype 2-3, are able to bind CEACAM significantly better than other strains. A secondary focus was the ability of Mx to form biofilms, the same genetically distinct group of Mx strains that demonstrated increased CEACAM binding also formed the strongest biofilms in the sub-population tested. Finally, these phenotypes were analysed after growth in conditions which encouraged phase variable expression of large autotransporters. We identified changes in expression of key cell surface adhesins, Hag and UspA2, resulting in phenotypes associated with increased persistence and cellular adhesion

Conclusion:

Results indicate a significant increase in both CEACAM1 binding and biofilm formation in this genetically distinct group of strains. We also provide evidence of phase variable expression of UspA2V as well as an increase in biofilm formation after phase variation of surface adhesins.

B276

The effect of glucose on motility and invasion in *Streptococcus gallolyticus* subspecies *gallolyticus* and related species

Adam Ralph ORCID iD, Jennifer Mitchell

University College Dublin, Dublin, Ireland

Abstract

Streptococcus gallolyticus subspecies *gallolyticus* (*Sgg*) is an opportunistic gut pathogen that enters the bloodstream via colorectal cancer (CRC) tumours causing subsequent bacteraemia and infective endocarditis. *S. gallolyticus* subspecies *pasteurianus* (*Sgp*) and *S. lutetiensis* (*Sl*) are closely related gut streptococci capable of causing bloodstream infections yet they are not associated with CRC.

We recently discovered that *Sgg* displays twitching motility and CRC cell invasion that is inhibited when the bacteria are grown in excess sugar. We hypothesised that *Sgp* and *Sl* might be less motile given their lack of association with bloodstream infections in cancer patients. However, experiments showed that they are more motile and less inhibited by excess sugars.

Following on from this, HT-29 and HCT-116 CRC cells were infected with all three streptococci grown in the presence/absence of glucose (0.5%). Glucose significantly reduced the ability of all three streptococci to invade both CRC cell lines. However, *Sgg* invades HCT-116 to a greater degree than HT-29, while *Sgp* exhibits the opposite result. This is notable given that HCT-116 cells are a more aggressive cancer cell line compared to HT-29 cells.

Contrary to our initial hypotheses, *Sgg* motility is more tightly regulated than *Sgp* and *Sl* in the presence of glucose and its invasion is dependent on how aggressive the tumour cells are, whereas *Sgp* and *Sl* invasion is not. This is an exciting finding and we have carried out comparative genomics in an effort to reveal the relationship between *Sgg* and CRC that is absent in closely related species.

B277

Characterising the Proteomes of *Salmonella* Outer Membrane Vesicles Under Intestinal Conditions

Alastair Scott ORCID iD[1], Mark Stevens ORCID iD[2], Garry Blakely[1], Prerna Vohra ORCID iD[1]

[1]University of Edinburgh, Edinburgh, United Kingdom. [2]The Roslin Institute, Edinburgh, United Kingdom

Abstract

Salmonella enterica is a bacterial pathogen of global importance. Serovars of *S. enterica* cause disease ranging from acute enteritis (e.g.: *S.* Typhimurium in humans, cattle, and pigs) to systemic disease (e.g.: *S.* Dublin and *S.* Choleraesuis in cattle and pigs, respectively, and occasionally humans). Like other Gram-negative bacteria, *Salmonella* produces outer membrane vesicles (OMVs) that participate in pathogenesis by fusing to host cells to deliver virulence factors that aid infection and manipulate the immune system. Protein packaging into OMVs can be influenced by environmental conditions including those found in the gut. Here, we characterised changes in the OMV proteomes of *S*. Typhimurium, *S*. Dublin, and *S*. Choleraesuis strains of defined virulence when cultured in laboratory conditions (lysogeny broth) compared to intestinal levels of bile (1%) from cattle and pigs to identify serovar-specific differences that relate to the type of disease they cause. OMVs were purified from late exponential phase cultures using density gradient ultracentrifugation and quantified by nanoparticle tracking analysis. Periplasmic proteins were also prepared at the same growth phase by cold-osmotic shock. Data-independent acquisition mass spectrometry revealed both serovar- and condition-specific differences in OMV proteomes. For all serovars, additional periplasmic proteins were packaged into OMVs in the presence of bile and more cytoplasmic proteins were found suggesting a loss of selective periplasmic packaging and possible protein leakage across the inner membrane, respectively. Furthermore, 40 proteins were considerably more abundant when the bacteria were cultured in bile. Investigations into how changes in OMV protein packaging may affect virulence are ongoing.

B278

Growth of *Paracoccus aminovorans* is restricted by small molecules and human gut isolates

Kiran Heer[1,2], Nandita Sharma[1,2], Surajit Basak[3], Saumya Raychaudhuri[1]

[1]CSIR-IMTECH, CHANDIGARH, India. [2]AcSIR, Ghaziabad, India. [3]NICED-ICMR, Kolkata, India

Abstract

Paracoccus aminovorans is Gram-negative, rod shaped and obligate aerobe bacterium that belongs to the genus *Paracoccus.* Members of this genus play a crucial role in the soil microbiome and contribute immensely to the process of microbial remediation of contaminated soil. Intriguingly, *Paracoccus aminovorans* is also a member of the human gut microbiota, and it exerts a positive influence on the survival of *Vibrio cholerae*, thus contributes in disease progression. As *Paracoccus aminovorans* aids in the establishment of cholera pathogenesis in the host gut, we wanted to control the growth of this abettor by exploiting some non-antibiotic therapeutic strategies. In this regard, we selected recent therapeutic approaches that are effective against *Vibrio cholerae* under various in vitro conditions. One such approach is where a combination of glucose and probiotic strains effectively restricts *Vibrio cholerae* pathogenesis, we exploited similar synbiotic approach with different gut commensals against *Paracoccus aminovorans*. In the present study, we isolated commensal bacteria from the fecal sample of a healthy donor and characterized those isolates by molecular taxonomy and genome sequencing. One isolate, *Weissella confusa*, in the presence of glucose, appeared to restrict *Paracoccous aminovorans* growth in co-culture growth condition. In the second approach, we exploited two small molecules, namely epigallocatechin gallate (EGCG) and L-ascorbic acid (LA), and both are effective against *Vibrio cholerae*. We observed both these molecules also exert a strong growth inhibitory effect on *Paracoccus aminovorans.* Collectively, the present study suggested that non-antibiotic strategies that are effective against *Vibrio cholerae* are equally effective against *Paracoccus aminovorans.*

B279

Role of Type II MLA system towards Campylobacter jejuni Outer Membrane Vesicle biogenesis

Subhadeep Gupta ORCID iD[1], Amirul Mallick ORCID iD[2], Ozan Gundogdu ORCID iD[1]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom. [2]Indian Institute of Science Education and Research Kolkata, Mohanpur, India

Abstract

Campylobacter jejuni is a major cause of diarrhoeal diseases in humans, with poultry as the primary transmission source. While lacking classical virulence factors, *C. jejuni* uses nano-sized outer membrane vesicles (OMVs) for cargo transport and cell communication. In a murine model, wild-type OMVs (nOMVs) play a bi-functional role, promoting pathogenesis and influencing immune responses. Recent evidence suggests that OMV biogenesis, regulated by the conserved Maintenance of Lipid Asymmetry (MLA) pathway, may be key in determining host responses. The MLA system in *C. jejuni*, a type II system, is genetically distinct and lacks the periplasmic carrier MlaC. Instead, MlaD, with a coiled-coil domain, spans the periplasm, while the uncharacterized protein MlaG is associated with the outer membrane. This system represents a novel class of ABC transporters that likely spans both membranes and the periplasmic space, but how this MLA system is important in *C. jejuni* OMV production remains elusive. To this end, we have undertaken in-depth functional characterisation of the type II MLA system toward OMVs biogenesis and biophysical character to develop a future road map to mitigate the problem associated with persistent colonisation of *C. jejuni* in chickens. In line with this, we have generated mutants of *C. jejuni* by deleting the *mlaA* and *mlaC* genes in the isogenic background. Next, we isolated and characterised the OMVs from the Δ*mlaA* and Δ*mlaC* of *C. jejuni*. Taken together, these findings illustrate how the functionality of the type II MLA system can modulate *C. jejuni* OMV biogenesis and its physical characteristics.

B280

Synergistic Enhancement of Antibiotic Efficacy Against Biofilms with Cold Plasma

Thomas Thompson[1], Jordanne-Amee Maybin[1], Ross Duncan[1], Paula Bourke[2], Noreen Hickok[3], Theresa Freeman[3], Brendan Gilmore[1]

[1]School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom. [2]School of Biosystems and Food Engineering, University College Dublin, Dublin, Ireland. [3]Sidney Kimmel Medical College of Thomas Jefferson University, Philadelphia, USA

Abstract

The persistent challenge of antibiotic-resistant biofilm infections calls for novel treatment approaches. This study investigates the use of cold atmospheric plasma as a pre-treatment to enhance antibiotic efficacy against *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms. Cold plasma pre-treatment induces temporary disruptions in biofilm cell membranes, increasing permeability, and reducing biofilm tolerance to antibiotics.

Our approach focused on varying plasma exposure times (30, 60, and 120 seconds) to determine optimal pre-treatment durations that maximise antibiotic susceptibility. Biofilms were grown under static and shaking conditions to assess the impact of biofilm morphology on treatment efficacy. Using transcriptomic analysis, we examined gene expression changes following CAP treatment, identifying complex cellular responses linked to enhanced antibiotic susceptibility, including alterations in membrane integrity and metabolic activity.

Results demonstrate that plasma pre-treatment significantly reduces biofilm tolerance, achieving a notable reduction in minimum biofilm eradication concentrations (MBECs) for antibiotics like ciprofloxacin. The study reveals that both biofilm type and plasma exposure duration are critical factors in maximising the synergistic effect, suggesting a customisable plasma-antibiotic approach for different biofilm structures.

B281

Elucidating the role of glycobiology at the oral host-pathogen interface

Samie-Jo Terry[1], Graham Stafford[1], Daniel Lambert[1], Radoslaw Kozak[2]

[1]University of Sheffield, Sheffield, United Kingdom. [2]Ludger, Oxford, United Kingdom

Abstract

A hallmark of periodontal disease is the expansion of the red complex, which includes *Tannerella forsythia* (Tf). Sialic acids (Sias) are terminal residues of glycoconjugates that, once removed by sialidases, Glycosyl Hydrolase 33 (GH33) family of carbohydrate-active enzymes, reveal adhesion epitopes and carbon sources. The Tf genome contains two GH33 enzymes NanH, and BFO_0701. This project aims to understand how Tf utilises and alters the Sia landscape in the oral cavity and the roles of NanH and BFO_0701 in this endeavour. We employed HILIC-UPLC, cytokine bead arrays (CBA) and invasion assays to determine oral gingival keratinocytes surface glycan alterations after Tf exposure, and its relation to bacterial invasion and host immune response. HILIC-UPLC indicates terminal Sia removal. However, intermediate surface N-glycan structures were absent in the mass spectrometry profiles, suggesting that Tf cleaves the underlying structures en-bloc or sequentially. CBA reveals that NanH contributes to a proinflammatory environment and proinflammatory cytokine release in oral gingival keratinocytes. His-tagged BFO_0701 was purified and studied using enzymatic assays. Substrate range and kinetics were investigated, BFO_0701 has a Km of 98.58uM against MU-NANA compared to 6.3uM for NanH. NanH and BFO_0701 are active against MU-KDN with Km of 3.753uM and 29.26uM respectively. For both substrates, BFO_0701 displays positive cooperativity, and enzyme activity can be inhibited by classical sialidase inhibitors. This study contributes to understanding Tf in the context of host-pathogen and pathogen-pathogen interactions, we suggest Tf can act upon a greater range of Sias than previously studied.

B282

Understanding the functional and genetic diversity of a collection of lytic bacteriophages for screening against Pseudomonas aeruginosa clinical isolates.

Anna Richmond ORCID iD[1], Rama Bhatia ORCID iD[1], Mark Szczelkun ORCID iD[2], Stineke van Houte ORCID iD[1]

[1]University of Exeter, Exeter, United Kingdom. [2]University of Bristol, Bristol, United Kingdom

Abstract

Bacteriophages (phages) are viruses that specifically target bacteria, they are commonly highly host-specific and represent an alternative to antibiotics. Bacteria carry defence systems against phages. These defence systems can evolve to provide resistance against specific phages. The importance of such systems is not currently fully understood.

We generated a collection of over 80 lytic phages that target the multi-drug resistant *Pseudomonas aeruginosa*. These phages were analysed for their taxonomic, functional, genetic and proteomic diversity. This diversity covers phage entry into cells, phage metabolism, and genetic similarity, all potential targets for bacterial defence systems.

This phage collection will be screened for infectivity against a diverse range of *P. aeruginosa* clinical isolates. These clinal isolates have been sequenced and analysed for defence systems. Therefore, this high-throughput screening will establish the effectivity of a range of phage profiles against different defence systems in their natural host. This work is part of the sLoLa Multi-Defence project and will further the understanding of the relationship between defence systems and phage resistance, which will be crucial in establishing successful phage therapy for *P. aeruginosa* infections.

B283

Use of 3D intestinal cultures as platforms for the discovery of compounds with anti-*Cryptosporidium* activity

Yuanxi Shen ORCID iD[1], Carrie Duckworth ORCID iD[2], Barry Campbell ORCID iD[1]

[1]Infection Biology & Microbiomes, University of Liverpool, Liverpool, United Kingdom. [2]Molecular & Clinical Cancer Medicine, University of Liverpool, Liverpool, United Kingdom

Abstract

Cryptosporidium can cause diarrhoea in both humans and animals, with limited treatment options. Drug screening targeting *Cryptosporidium* has been limited by suitable epithelial cell models, which typically fail to support the full life cycle of the parasite. Physiologically relevant 3D models, that recapitulate the gut epithelium, would provide a more ideal platform. Following encystation of *C. parvum* oocysts with sodium hypochlorite, we infected two 3D intestinal epithelium models, (1) murine intestinal crypt-stem cell enteroid-derived monolayers, and (2) 3D HCT-8 cell-line aggregates (generated in rotary vessels), compared with 2D HCT-8 monolayers. Anti-*Cryptosporidium* therapeutics, such as Halofuginone (HF) and Nitazoxanide (NTZ) were tested during 3h of infection and post 3h infection and were followed for 48h. Cytotoxicity, cytokine responses (IL-8, IFN-γ and IL-10) and qPCR for *Gapdh* transcripts to quantify *C. parvum* were measured.

In 2D HCT-8 cells, 24-72h infection led to prominent increases in IL-8 (up to 2000 pg/mL). The 3D HCT-8 aggregates and enteroid monolayers effectively supported *C. parvum* infection. HF (tested at 0.04-25µM) effectively reduced parasite load when administered during and post 3h infection, with minimal cytotoxicity during infection, but higher cytotoxicity post infection, across all 3 models. However, NTX was only effective at 20µM administered during 3h infection in 2D HCT-8 cells, and at 1-20µM post infection but with significant host cell cytotoxicity. NTX was not effective in the 3D models. In summary, 3D aggregates and enteroid cultures provide an optimal environment for studying *C. parvum* infection and offer a valuable platform for evaluating anti-*Cryptosporidium* drug effectiveness.

B284

Effects of ZnO on the presence of enterotoxigenic Escherichia coli and Rotavirus in weaner piglets

Andrew Bease[1], Samuel Connelly[2], Nicky Craig[1], Jade Davies[2], Muna Anjum[2], Manal Abu Oun[2], Christina Vrettou[1], Ilias Kyriazakis[3], Mark Stevens[1], Abel Ekiri[4], Roberto La Ragione[4], Nick Wheelhouse[5], Sam Beechener[6], Sadie Douglas[7], Amy Taylor[7], Deborah Hoyle[1]

[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom. [2]Animal and Plant Health Agency, Weybridge, United Kingdom. [3]Queen's University of Belfast, Belfast, United Kingdom. [4]University of Surrey, Guildford, United Kingdom. [5]Edinburgh Napier University, Edinburgh, United Kingdom. [6]Scotland's Rural College (SRUC), Inverness, United Kingdom. [7]AB Neo, Ripon, United Kingdom

Abstract

Post-weaning diarrhoea (PWD) is an important disease syndrome of weaner piglets, resulting in weight loss, morbidity and mortality. The primary causative agent is enterotoxigenic *Escherichia coli* (ETEC), with co-infecting pathogens, particularly Rotavirus, contributing to disease susceptibility. Until recently, Zinc oxide (ZnO) was added to feed at pharmacological levels (zinc, 2500 ppm), to reduce PWD and boost growth. However, zinc is thought to promote antimicrobial resistance (AMR) through shared resistance mechanisms and is of environmental concern due to pollution from zinc in slurry. Supplementation of feed with zinc above 150 ppm was therefore prohibited in the UK from June 2024. Producers anticipate PWD will become harder to manage, potentially increasing antimicrobial usage.

The aim of this study was to investigate the impact of ZnO withdrawal on the presence of key PWD pathogens, AMR and the microbiome in weaner piglets. Here, faecal samples collected through a longitudinal study on 24 commercial UK pig herds during ZnO withdrawal between 2022-2024 were tested by multiplex PCR for ETEC-specific fimbrial (*faeG*, *fanA*, *fim41A*, *fasA*, *f17A*, *fedA*) and toxin (*eltA*, *sta1, stx2e*) genes and by reverse transcription PCR for Rotavirus species A, B, C and H. Herd and sample level prevalence were determined for all targets, and for *E. coli* pathotype, according to combination of fimbrial and toxin signal. Relationships between the presence of ETEC gene and Rotavirus A, B, C or H PCR signals, together with key herd metadata variables, such as vaccine usage, were determined using tabular methods, tetrachoric correlation and multivariate statistical analyses.

B285

Synthetic wound fluid enhanced the antimicrobial susceptibility and altered the biofilm formation ability of chronic wound bacterial isolates of *P. aeruginosa* and MRSA

Charlotte R. Morgan, Aleyna Yarranton, Lydia C. Powell

Swansea University Medical School, Institute of Life Sciences 1, Singleton Park, Swansea, United Kingdom

Abstract

Chronic non-healing skin wounds are a significant cause of morbidity and mortality in patients. Bacterial biofilm-related infections formed within these wounds impair tissue healing, resist conventional treatment and harbour multi-drug resistant bacteria. Therefore, there is an urgent need to gain a greater understanding of how bacterial biofilms form within chronic wounds to enable the development of alternative therapies. Chronic wound bacterial isolates of *Pseudomonas aeruginosa* PAO1 and Methicillin-resistant *Staphylococcus aureus* (MRSA) 1004a were grown in two recipes of synthetic wound fluid (SWF), comprised of either fetal bovine serum (FBS) or bovine serum albumin (BSA) in comparison to a standard laboratory media, Mueller Hinton Broth (MHB). SWF containing FBS supported bacterial growth of both *P. aeruginosa* and MRSA, while SWF containing BSA reduced growth. Unexpectedly, MRSA grown in SWF containing FBS exhibited increased antimicrobial susceptibility to colistin in comparison to MHB, resulting in a reduced minimum inhibitory concentration (MIC) value from 256µg/ml to 16µg/ml. The biofilm formation ability of the bacterial isolates in SWFs were characterised by confocal laser scanning microscopy with COMSTAT analysis, which revealed significant alterations in *P. aeruginosa* and MRSA biofilm biomass and dead/live bacterial ratio in comparison to MHB ($p < 0.05$). Atomic force microscopy and electrophoretic light scattering assays revealed alteration of the cellular mechanical properties and a less negative bacterial surface charge when grown in SWF containing FBS, in comparison to MHB ($p < 0.05$). This greater understanding will provide new insights into how bacterial biofilm related infections are established within chronic wound beds.

B286

Phage therapy for the treatment of Non-Cystic Fibrosis Bronchiectasis in a Norfolk Patient Population: Insights from the BronchoPhage Study.

Hannah V. Pye ORCID iD[1,2], Catherine Dominic ORCID iD[3,4], Revathy Krishnamurthi ORCID iD[1,2], Gemma Beasy[1,2], Jennifer Ahn-Jarvis ORCID iD[1,2], Antonietta Hayhoe[1,2], Eleanor K. Mishra ORCID iD[3,4], Mark Pasteur[3], Evelien M. Adriaenssens ORCID iD[1,2]

[1]Quadram Institute Bioscience, Norwich, United Kingdom. [2]Centre for Microbial Interactions, Norwich, United Kingdom. [3]Department of Respiratory Medicine, Norfolk and Norwich University Hospitals Foundation Trust, Norwich, United Kingdom. [4]Norwich Medical School, University of East Anglia, Norwich, United Kingdom

Abstract

Non-cystic fibrosis bronchiectasis (NCFB) is a chronic respiratory disease characterised by dilated airways and persistent sputum production, resulting in recurrent bacterial infections often dominated by *Pseudomonas aeruginosa*. NCFB affects over 200,000 people in the UK, yet antibiotic treatment is limited due to the rise in antimicrobial resistance. As treatment with bacteriophages has been shown effective for infections in cystic fibrosis patients, this study aims to explore the use of phage therapy (PT) in NCFB.

The BronchoPhage study is investigating the genomic diversity of *P. aeruginosa* within a NCFB patient cohort at the Norfolk and Norwich University Hospital, and their suitability for PT. A total of 116 patients with confirmed *Pseudomonas*-associated NCFB were considered, with 49 providing baseline sputum samples. A subset of participants also provided sputum samples before and after receiving antibiotic treatment during exacerbation. Diverse *P. aeruginosa* isolates were collected from sputum and sequenced. The phylogenetic relationship between inter- and intra-patient strains was assessed. The host range of a pre-existing *P. aeruginosa* phage collection was evaluated against patient-derived *P. aeruginosa* strains, highlighting the need for targeted phage isolation. The MIC of commonly prescribed antibiotics (Gentamicin, Colistin, Meropenem, Chloramphenicol, Ceftazidime and Piperacillin-Tazobactam) revealed varied resistances.

PT is a promising treatment option for NCFB, however, to unlock its full potential further research into how phages can effectively target and eliminate infections within the respiratory tract and interact with the resident lung microbiota is needed. Only by addressing these challenges can PT evolve into a reliable and transformative treatment option for NCFB.

B287

Imaging the dynamics of DNA repair proteins in an intracellular pathogen.

Anju Kudhail, Fiona Sargison, Amy Moores, Elliott Haywood, Stephan Uphoff

Department of Biochemistry, University of Oxford, Oxford, United Kingdom

Abstract

Macrophages are a key cell type of the innate immune system, which aim to kill bacteria following uptake by phagocytosis. Intracellular bacterial pathogens are able to survive and replicate within macrophages, but it is not understood how they resist killing by genotoxic agents present in the phagosome. To better understand this, we are optimising a super-resolution microscopy method to image the intracellular pathogen *Salmonella enterica* serovar Typhimurium (*S*. Tm) within live macrophages. Using this method, we can track the activity of single DNA repair proteins, which reflects the repair of DNA damage inflicted by the macrophage. This enables us to characterise the bacterial factors which aim to safeguard the *S*. Tm genome, and the macrophage factors which challenge this. Together, this will provide novel insights into the complex host-pathogen interactions at play between intracellular bacteria and their macrophage host cell.

B288

Combination Effect of Laser Irradiation and Sodium Hypochlorite on Killing of Disinfectant-Resistant Staphylococcus aureus Isolated from Wounds

Mohemid Al-Jebouri ORCID iD

Kirkuk, Kirkuk, Iraq

Abstract

Background: Laser as a new phototherapeutic approach was proposed its basic theory since beginning of last century. It was used in wound disinfections particularly with antibiotic as well as disinfectant-resistant bacterial pathogens. Methodology: The laser was a helium-neon (He/Ne) gas type with a measured output at 5 mW (Laser Becaon,I.N.C. Michigan, USA) was used in the present study.Toludine blue 0 and providine – iodine as photosensitizers were used. Hypochlorite (Britain Drug Home) was utilized. Selected disinfectant-resistant strains of *S. aureus* were tested.Using minimal inhibitory concentration of different antibiotics was assessed and its effect on total viable counts (TVC's) of strains was concluded. Results: The effect of laser,sodium hypochlorite and laser-sodium hypochlorite on selected strains of *S. aureus* was seen. There was significant decreases in TVC's after exposure to laser – sodium hypochlorite combination compared to laser, providine-iodine or sodium hypochlorite. Other changing patterns of resistance to antibiotics as well as disinfectants and heavy metals were clearly observed. Conclusions: High significant decreases were achieved in antibiotics resistance.Minimal inhibitory concentrations of chemical disinfectants, heavy metals, and antibiotics after exposure to laser-providine-iodine-sodium hypochlorite combination of disinfectant-exposed pathogen were dramatically changed depending on types of combinations.

B289

Investigation into the role of biofilm in *Staphylococcus aureus* mastitis

Christine S. Grant, Amy C. Pickering, Nicola Lynskey, J. Ross Fitzgerald

Roslin Institute, Edinburgh, United Kingdom

Abstract

Staphylococcus aureus is a leading cause of mastitis in dairy cattle with major economic and animal welfare consequences. *S. aureus* has the capacity to form biofilms that promote survival during infection and resilience to anti-microbial activity. Bovine strains of *S. aureus* associated with mastitis evolved via host-switch events from humans. Through investigations into the evolutionary adaptation of *S. aureus* to the dairy niche, we have identified a novel milk-associated biofilm phenotype. The biofilm is produced in the presence of bovine milk on materials used in milking equipment including steel, silicone, and rubber, and are more robust than classical *S. aureus* biofilms. We have discovered that expression of the extracellular protease aureolysin is required for the milk-associated biofilm phenotype. Preliminary scanning electron microscopy analysis has facilitated visualisation of the biofilm matrix, but the major structural components are unknown. An array of gene deletion mutants deficient in production known biofilm-associated factors including aureolysin, the intercellular adhesin, Sortase A and Biofilm-associated protein have been constructed in multiple bovine *S. aureus* lineages. The mutants will be compared to their wild type strains in a series of functional assays to identify the bacterial factors required for the milk-associated biofilm. Overall, these data will provide new insights into a novel biofilm phenotype that may promote the transmission and pathogenesis of mastitis.

B290

Using a genomic approach to investigate the adaptability of *S. aureus* clinical strains to an iron restricted environment

Kate Kearney[1,2], Elizabeth Ledger[1,2], Mario Recker[3], Ruth Massey[1,2,4]

[1]School of Microbiology, University College Cork, Cork, Ireland. [2]APC Microbiome Ireland, University College Cork, Cork, Ireland, Cork, Ireland. [3]Centre for Ecology and Conservation, University of Exeter, Penryn Campus, Exeter, United Kingdom, Exeter, United Kingdom. [4]School of Cellular and Molecular Medicine, University of Bristol, Bristol, United Kingdom, Bristol, United Kingdom

Abstract

Staphylococcus aureus is an important opportunistic pathogen, with the ability to cause a wide range of infections ranging from mild skin and soft tissue infections (SSTI) to life-threatening bacteraemia, which has a mortality rate between 20-30%.

Iron is an essential micronutrient required for *S. aureus* growth and colonisation. *S. aureus* employs various iron acquisition mechanisms to overcome iron sequestration from its host, such as high-affinity iron chelators called siderophores. However, the mechanisms utilised by *S. aureus* to acquire iron from its environment are not fully known. Our work aims to address these gaps and better understand how *S. aureus* acquires iron from its host.

We optimized a high-throughput assay to compare the effect of iron limitation on a set of 266 clinical *S. aureus* isolates. We have shown that iron usage varies across MRSA and MSSA strains isolated from colonizing sites, bloodstream infections and SSTI.

On comparison of our human clinical data to data obtained from the iron limitation assay a significant link was observed between growth defects in an iron restricted media and the urogenital tract as an entry point and focus of infection.

Future work will use functional genomics in the form of a genome wide association study to identify single nucleotide polymorphisms associated with the susceptibility of isolates to an iron restricted environment.

In conclusion, our work will use a genomics approach to enhance understanding of how *S. aureus* adapts to the host environment.

B291

Barking Up The Right Tree: *Ficus natalensis* Bark Cloth as a Sustainable Antimicrobial Fabric for Use in Wound Dressing Technology

Jack Buckley

Manchester Metropolitan University, Manchester, United Kingdom

Abstract

In 2008, the production of bark cloth from the Mutuba tree (*Ficus natalensis*) was dubbed an "intangible cultural heritage of humanity" by UNESCO. Bark cloth is a non-woven fibrous material that has been arduously and skilfully crafted by the Baganda people of Southern Uganda since at least the 13th century and reflects the identity of the community. Five other species of the *Ficus* genus have also been investigated. A plethora of *S. aureus* strains, as well as, several staphylococcal species and some commensal microflora were investigated as to their susceptibility to *F. natalensis.* The bark cloth was shown to exhibit excellent antimicrobial activity against S. aureus strain USA300 (an MRSA strain). After 24 hours of constant exposure, the bark cloth resulted in a seven-log reduction in MRSA viability. Scanning electron microscopy (SEM) was performed to provide further details regarding the antimicrobial mechanism of activity of the bark cloth. The imaging revealed bacterial attachment and biofilm formation on the fibrous structure of the bark cloth. Apparent morphological aberrations to the cell ultrastructure such as invaginations and perforations were observed. Such phenotypic alterations highlight a bactericidal, contact-kill effect against MRSA. Antibiofilm activity has also been noted, with *F. natalensis* showing an 87% reduction in staphylococcal biofilm using a modified CDC Biofilm Reactor method. Phytochemical profiling of *F. natalensis* has revealed a multitude of potentially active compounds. Flavonoids, saponins and diterpenes are particularly pertinent ingredients as they have known antimicrobial capacity.

B292

Single Molecule Imaging Reveals Molecular Effects of Ciprofloxacin on Intra-Macrophage *Salmonella*

Elliott Haywood[1], Fiona Sargison[1], Anju Kudhail[1], Mathew Stracy[2], Stephan Uphoff[1]

[1]Department of Biochemistry, University of Oxford, Oxford, United Kingdom. [2]Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom

Abstract

Salmonella enterica serovar Typhimurium is an intracellular pathogen capable of surviving within immune cells such as macrophages. Further, fluoroquinolone resistant *Salmonella* serovars are of increasing global concern. Both macrophages and fluoroquinolones such as ciprofloxacin are able to cause DNA damage, eliciting DNA damage response pathways and other stress responses that may allow survival of *Salmonella*. Despite this, the effects of ciprofloxacin or macrophage factors on *Salmonella* are often studied in isolation, with comparatively little known about the combined effects of these sources of damage. For example, the effects of ciprofloxacin on its cellular targets and on DNA repair pathways in intra-macrophage *Salmonella* is unclear. We used single molecule imaging approaches to investigate the effects of ciprofloxacin on its target proteins and downstream antibiotic-induced mutagenesis in intra-macrophage *S.* Typhimurium. We also measured the minimum bactericidal concentration and minimum duration of killing of ciprofloxacin against extra- and intra-macrophage *S.* Typhimurium to compare how the intracellular environment affects antibiotic efficacy. Given *Salmonella* invades epithelial cells and are regularly phagocytosed by tissue-resident macrophages, any understanding of ciprofloxacin treatment is incomplete without an appreciation of how ciprofloxacin affects intracellular bacteria. Our work therefore seeks to improve our understanding of how antibiotics and the harsh intracellular environment impact *Salmonella* in tandem, and how bacterial survival can occur in these conditions.

B293

Characterising the immunological potential of a novel B. pertussis adhesin, OtbAB.

Michael Gollan[1], Monica Gestal[2], Katelyn Parrish[2], Iain MacArthur[1]

[1]Northumbria University, Newcastle, United Kingdom. [2]Louisiana State University, Louisiana, USA

Abstract

As an obligate human pathogen, *B. pertussis* colonisation of respiratory tissues is a pre-requisite for disease; as a result, *B. pertussis* possesses a large repertoire of adhesins. With the current acellular vaccine, nasal carriage is potentiated via attenuation of the Th17 response (Dubois et al., 2021), resulting in a rise in the number of asymptomatic carriers who serve to perpetuate the disease. It is therefore of interest to prevent this initial adhesion to respiratory tissues and thus decrease transmission and consequent disease, whilst inducing a favourable immune response mimetic of that generated following natural infection.

We have identified a novel adhesin, OtbAB, constituted of Orphan B Toxins A and B, homologs of B subunits of AB toxins, without a cognate A subunit. We have found that these proteins retain their binding function, being important for adhesion of *B. pertussis* to respiratory epithelial tissues both *in vitro* and in a murine model. Of note, we found significant reductions in binding to nasal cavity tissues by ΔOtbA and ΔOtbB mutant strains (manuscript in review), suggesting their potential role in nasal colonisation and thus carriage.

To explore the application of these proteins, we investigated their immunological potential. Anti-OtbA and anti-OtbB IgG antibodies were found in human convalescent serum and therefore are generated against OtbA and OtbB during natural infection. We characterised the neutralisation and opsonisation potential of these antibodies to determine their role during infection and performed a vaccination challenge to observe their effect on inclusion to acellular vaccination in a murine model.

B294

SOMAmer technology to diagnose coronaviruses infection: A potential foundation for future rapid low-cost diagnostics

Jim Chadwick[1,2], Joseph Newman[1], Tiong Tan[3], Atherton Mutombwera[4], William James[2], Dalan Bailey[1]

[1]The Pirbright Institute, Woking, United Kingdom. [2]The Sir William Dunn School of Pathlogy, University of Oxford, Oxford, United Kingdom. [3]Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom. [4]Hutano Diagnostics Ltd, Oxford, United Kingdom

Abstract

Molecular recognition elements (MREs) for application in virology are based heavily on monoclonal antibodies despite high costs and production time. SOMAmers® as synthetic (Slow off-rate modified)aptamers offer a potential alternative. ssDNA SOMAmers® form via shape complementarity with subsections of target viral proteins. Following screening, ssDNA sequences against SARS-CoV-2 Spike were selected based on their high affinity and slow off-rate. Binding responses were first evaluated using an indirect ELISA system before kinetics and epitope binning were performed via surface plasmon resonance. We next examined whether SARS-CoV-2 Wuhan selected SOMAmers® could bind divergent coronaviruses from the alpha, beta and gamma genera, as well as more closely related sarbecoviruses from clades 1, 2, 3 and 5. By determining the binding properties of SOMAmers®, we can gain a deeper understanding of their potential to replace MAb in virus diagnostics. The panel of screened antigens represented coronaviruses of both veterinary and zoonotic relevance. We observed that, for some SOMAmers®, mutations in the spike protein receptor binding domains (RBDs) had no effect on binding, suggesting conserved epitopes and the potential viability of specific broadly-reactive MREs in lateral flow test applications. Furthermore, using competition-based approaches, distinct pairs of SOMAmers® were transferred to physical prototypes and further evaluated with detectable variants for cross-reactivity profiles. Considering pandemic preparedness, diagnostics are a key part of one health surveillance for emerging viral threats. Within scalable point-of-care testing, the viability of SOMAmers® as a robust alternative for detecting divergent coronaviruses offers a foundation for future diagnostic tools adaptable to emerging pathogens.

B295

Comparative analysis of dimorphic conversion in the *Sporothrix schenckii* complex

Hebatallah Mohammed, Albert Bolhuis

University of Bath, Bath, United Kingdom

Abstract

Sporotrichosis is a subcutaneous mycosis caused by the pathogenic species of the *Sporothrix schenckii* complex. Information about the pathogenicity distinction among these species remains limited. We tested this by measuring the phagocytosis percentage.

Members of the *S. schenckii* complex possess a unique ability of altering its morphological form between the filamentous (saprophytic) and the yeast (parasitic) forms. Hence, this conversion ability to yeast form is a primary determinant of pathogenicity.

We measured the efficiency of the filamentous-yeast switch under different conditions. We observed that higher pH and temperature, as well as nutrient abundance, play an important role in promoting this conversion. Interestingly, we observed that this conversion process is species-specific, being the highest in *S. brasiliensis*.

Furthermore, we investigated the percentage of yeast formation at different time intervals and after successive 4-day subcultures. We observed that *S. brasiliensis* showed the highest capability with a 96% of cells converted into the yeast form after only two subcultures. This is in stark contrast to, for instance, *S. schenckii S. Str,* which required ten successive subcultures before converting into 87.8% yeast cells.

In addition, the switch to the yeast form was confirmed by *ex vivo* analysis studies. We found that *S. brasiliensis* exhibited a greater ability to form a higher load of yeast cells adhering to the skin compared to other species.

To sum up, *S. brasiliensis*, the most virulent species in the *Sporothrix schenckii* complex as demonstrated in the macrophage model, showed higher capability and celerity of formation of the parasitic form.

B296

A surveillance study of *Cryptosporidium parvum* present in organic waste used as fertiliser in Ireland.

Emma Conlon[1], Paul Whyte[1], Declan Bolton[2], Gerald Barry[1]

[1]University College Dublin, Dublin, Ireland. [2]Teagasc, Dublin, Ireland

Abstract

In Ireland animal faeces, urine, and bedding generated on farms are spread on land as organic fertilisers to promote the growth of grass and certain commercial crops. Despite this practice being commonplace, its safety in Ireland has yet to be characterised from a microbial standpoint, particularly with regards to parasites.

There is a growing movement to reduce the use of chemical fertilisers and to reduce organic waste entering landfills, thus resulting in the land spreading of organic waste. Therefore, there is an urgent need to characterise this waste and identify pathogens which may pose a risk to public health. This assessment of the parasitic safety of organic waste in Ireland is part of a wider project entitled Safe Waste. This project aims to characterise and mitigate both chemical and microbial contaminants in organic waste in Ireland.

Preliminary data shows that 95% (n=84) of sheep samples and 81% (n=82) of cattle samples were positive for the presence of *Cryptosporidium parvum* DNA by nested PCR. The highest incidence of detection in sheep was seen in Summer (100%, n=12) and Winter (n=24), while Autumn (91%, n=22) showed the highest prevalence of *C. parvum* in cattle.

This research also focused on the development of a nested PCR assay for the sub-clinical detection of *C. parvum*. These results highlight the need for increased annual surveillance of *C. parvum* to inform the Irish agricultural sector on the prevalence of this pathogen and best practices for biosecurity.

B297

Combatting antimicrobial resistance: Small-molecule demethylase inhibitors as precision antimicrobial and anti-virulence agents.

Holly Ansell ORCID iD, Laila Ralif, Thomas Britten, Andrew Lewis, Jonathan Butler

Manchester Metropolitan University, Manchester, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a growing global public health issue, and it is estimated that deaths associated with AMR infections will exceed 10 million by 2050. Methicillin-resistant Staphylococcus aureus (MRSA) remains a priority pathogen for the development of treatment strategies. Evolving mechanisms of resistance within microbial populations continually render current therapeutics ineffective and threaten a global return to the pre-antibiotic era. Using small-molecule inhibitors (SMIs) designed against epigenetic bacterial processes, including methylation, represents an innovative approach to combatting AMR by modulating key bacterial virulence traits including biofilm formation and toxin production. Preliminary antimicrobial susceptibility screening identified several lead candidate demethylase SMIs which demonstrated activity at <6.25µM against several clinically relevant species of MRSA. Anti-biofilm activity of one lead compound was observed to inhibit formation of biofilms by 76% at 1.5 µM and eradicate 94.61% of established 24 hour biofilms at 200 µM. A 23% reduction in bacterial mediated haemolysis was observed in cells exposed to sub-inhibitory concentrations. q-RT PCR and RNA sequencing analysis in bacterial cells exposed to sub-lethal concentrations revealed regulatory changes in several key bacterial virulence factor related genes, including biofilm formation and oxidative stress repair. Research is now focused on validating the SMI bacterial target site and identifying global SMI-mediated proteomic changes to further elucidate the role of methylation via MALDI-MS and global protein methylation profiling. This research represents a major advance in the search for novel antimicrobial agents which target essential bacterial processes beyond those associated with traditional antibiotics.

B298

Dynamism of the CD9 interactome during staphylococcal and meningococcal infection

Paige A Wolverson, Isabel Fernandes Parreira ORCID iD, Jonathan G Shaw, Luke R Green

University of Sheffield, Sheffield, United Kingdom

Abstract

Bacterial adhesion to epithelial cells is often a critical first step in pathogenesis, with different bacterial species employing diverse mechanisms to achieve this. We have previously demonstrated that interference of CD9, a human tetraspanin, reduces bacterial adherence to epithelial cells by 50%. Although CD9 does not act as a direct receptor, it coordinates partner proteins into CD9-enriched microdomains exploited by bacteria for adherence, however, the complete CD9 interactome remains unidentified.

Using a novel CD9 proximity-labelling model, we identified a vast and diverse interactome, with 1,837 significantly enriched proteins over four hours. Known CD9 interactors and bacterial adherence receptors (CD44, CD46, CD147), were enriched. Interactome proteins were associated with key cellular pathways such as cell adhesion, ECM-receptor interactions, endocytosis, and tight junction integrity. Further, we demonstrated that infection with *Neisseria meningitidis* and *Staphylococcus aureus* revealed a dynamism within the CD9 interactome. Compared to uninfected cells, meningococcal infection uniquely enriched a further 13 proteins, while staphylococcal infection exhibited fewer alterations, demonstrating that different bacteria possess unique adherence requirements during CD9-mediated infections. Absence, or interference with CD9 using CD9-derived peptides, universally reduced bacterial adherence, highlighting the role of CD9 as an organizer of bacterial adhesion platforms. However, knockdown of CD44 and CD147 reduced adherence of *S. aureus* and *N. meningitidis*, respectively, in a CD9-dependent manner.

This study presents the first full characterisation of the CD9 interactome, and its bacterial species-specific dynamic remodelling during infection, establishing CD9 as a universal organiser of bacterial adhesion platforms which can be stopped using specific CD9-derived peptides.

B299

The antimicrobial activity of ethylenediaminetetraacetic acid (EDTA), against *Burkholderia* species

Mousomi Chakravorty, James Barnett ORCID iD

Birmingham City University, Birmingham, United Kingdom

Abstract

The *Burkholderia cepacia complex* (*Bcc*) consists of at least 24 species of bacteria that have the capacity to cause opportunistic infections in vulnerable individuals. They were first recognised as human pathogens in the 1980s, when they were identified as the cause of severe respiratory infections in people with cystic fibrosis (CF). Bcc infections often lead to a significant decline in lung function, and in rare cases can lead to the development of a life-threatening "cepacia syndrome," characterised by bacteraemia, and severe pneumonia. *Bcc* bacteria have significant intrinsic resistance to several antibiotics, meaning infections are difficult to clear. *Bcc* bacteria have wider medical significance, with infections also occurring in people with chronic granulomatous disease, as well as nosocomial infections that have been linked to contaminated medical supplies. There is thus an urgent need for more effective drugs to treat these infections and improve patient outcomes.

There is growing interest in the potential of metal chelation as a novel approach to tackle multidrug resistant bacterial infections. Here we report for the first time, the antimicrobial activity of ethylenediaminetetraacetic acid (EDTA), against 3 distinct species of *Burkholderia*. Significantly, we have found that *Burkholderia cenocepacia* and *Burkholderia multivorans*, that cause the majority of Bcc infections in CF patients, are able to tolerate higher concentrations of EDTA, than *Burkholderia gladioli*. *B. gladioli* does not belong to the Bcc but is another opportunistic pathogen of clinical significance.

B300

Studies on CEACAM1 engagement by bacterial pathogens.

Vitalija Pavlovaite

University of Bristol, Bristol, United Kingdom

Abstract

Carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) are critical glycoproteins in immune defence, facilitating cell interactions and pathogen recognition. However, certain pathogens, including *Fusobacterium nucleatum*, which is linked to periodontal disease and colorectal cancer—exploit CEACAMs to invade host tissues and avoid immune detection. Specifically, *F. nucleatum* binds to CEACAM1 on epithelial cells, supporting bacterial colonisation, promoting inflammation, and contributing to disease progression. To investigate this binding process, various techniques were employed, including infection assays on tissue cultures (HeLa Neo, HeLa CC1, and HT29 cell lines), light microscopy imaging, and protein analysis through western and dot blotting. These methods help to clarify the molecular interactions between CEACAM1 and *F. nucleatum*, providing insights into the mechanisms underlying infection and inflammation. This research suggests new pathways for understanding the bacterial adhesion process. It lays the groundwork for developing possible therapeutic strategies that are aimed at disturbing these interactions to mitigate infection-related disease progression and guiding future vaccine design.

B301

Typhoid Through Time: Exploring 20 years of Enteric Fever in England and Wales

Alice M. Nisbet[1], Emma V. Waters[1], David Powell[2], Iman Mohamed[3], Neville Q. Verlander[4], Gemma C. Langridge[1], Marie A. Chattaway[2]

[1]Microbes in the Food Chain, Quadram Institute Bioscience, Norwich, United Kingdom. [2]Gastrointestinal Bacteria Reference Unit, United Kingdom Health Security Agency, London, United Kingdom. [3]Travel Health and International Health Regulations, United Kingdom Health Security Agency, London, United Kingdom. [4]Data Analytics and Surveillance, United Kingdom Health Security Agency, London, United Kingdom

Abstract

On average, 415 cases of enteric fever are reported annually in England and Wales, encompassing disease caused by *Salmonella enterica* serovars Typhi, Paratyphi A and B: the typhoidal *Salmonella* (TS). Here, data collected by UKHSA facilitated examination of patient metadata and bacterial genomes from cases between 2004-2023.

Over the last two decades, England and Wales reported 8,297 cases of enteric fever, with >90% being associated with foreign travel. *S.* Typhi was the dominant causative agent. Annual case numbers fluctuated, notably dropping during the COVID-19 pandemic, before rising in 2023 to surpass pre-pandemic levels. Infections were most prevalent among 21–30 year olds and >60% occurred in those from the most deprived communities.

S. Typhi and *S.* Paratyphi A infections were most associated with the visitation of friends/relatives in Pakistan or India, with individuals whose ethnic origins laid in these regions accounting for >80% of cases. *S.* Paratyphi B infections were instead associated with holidays to South America and Western Asia–with phylogenetic analyses distinctly separating isolates from different locations.

2.7% of cases examined failed to clear after 3 weeks, and thus were considered ‘carried’ infections – a poorly understood phenomenon. These isolates failed to cluster into specific genomic profiles; however statistical analysis highlighted significant links between carriage and patient age, infecting serovar and travel occurrence.

This work emphasises the need to study TS as individual pathogens and has identified groups at increased risk of acute and carried infections, thus providing guidance for future research efforts and public health action.

B302

Elucidating the Role of *Fusobacteria* in Carcinogenesis

Raisha Gibbs ORCID iD

University of Bristol, Bristol, United Kingdom

Abstract

The *Fusobacterium* genus comprises Gram-negative, obligate anaerobic bacteria that typically reside in the periodontium of the oral cavity, gastrointestinal tract, and female genital tract. The association of Fusobacterial *spp.* with colorectal tumours is widely accepted, with further evidence that this pathogen may also be implicated in the development of other malignancies. Fusobacterial *spp.* influence malignant cell behaviours and the tumour microenvironment in various ways, which can be related to the expression of multiple adhesins, including CbpF (CEACAM binding protein of *Fusobacteria*), Fap2 (fibroblast-activated protein 2) and FomA (Fusobacterial outer membrane protein A).

Wound healing assays were utilised to investigate the influence of Fusobacterial spp. on malignant cell behaviour. Results suggest that Fusobacterial *spp.* exert varying impacts on the behaviour of ovarian and colorectal cell lines, with the addition of co-culture suggesting increased migratory and proliferative capacity. These responses to Fusobacterial co-culture were further reflected in relation to the cell surface molecules which serve as the binding partners for Fusobacterial adhesins: glactose-β(1-3)-N-acetyl-d-galactosamine (Gal-GalNAc), carcinoembryonic antigen-related cell adhesion molecule (CEACAM) and vascular endothelial cadherin (VE-Cadherin). Results show significant alterations in cell surface molecule expression, demonstrated by microscopy.

Recent 3D culture methods are shown to influence the expression of these key cell surface molecules. Moreover, live microscopy reveals that the influence of Fusobacterial co-culture is more pronounced in 3D methods than in traditional 2D culture techniques.

Together, these results demonstrate that Fusobacterial *spp.* may play pivotal roles in mediating the behaviour of malignant cells in both2D and 3D cell culture.

B303

Promising Novel Vaccine Antigen Homologs Against Antibiotic-Resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in an Acute Pneumonia Model.

Nouran Rezk ORCID iD[1], Julen Tomás-Cortázar ORCID iD[1], Chaoying Ma ORCID iD[1], Avinash Shende[2], Irene Jurado-Martín ORCID iD[1], Siobhán McClean ORCID iD[1]

[1]University College Dublin, Dublin, Ireland. [2]College of Liberal Arts, Penn State University, Pennsylvania, USA

Abstract

Acinetobacter baumannii and *Pseudomonas aeruginosa* are major antibiotic-resistant pathogens responsible for hospital-acquired infections. Classified by the World Health Organization (WHO) as "critical" and "high" priority pathogens, respectively, they urgently require novel treatments. Although vaccines show promise in combating infections, no licensed vaccines are available.

We have identified novel proteins involved in the host-cell attachment of *P. aeruginosa* and *A. baumannii* to lung cells, which are homologous in both pathogens. This study evaluates the protective efficacy and cross-reactivity of antigens, *A. baumannii* AgN and *P. aeruginosa* AgN.

Mice immunised with purified *A. baumannii* AgN adjuvanted with Sigma Adjuvant System (SAS) and subsequently challenged with acute *A. baumannii* pneumonia exhibited significant reductions in bacterial lung colonisation (0.74-$\log_{10}$ CFU, $p<0.0102$) and spleen dissemination (0.9-$\log_{10}$, $p<0.0426$), along with high total IgG and IgG1 antibody titres (1: 62,500 and 1:78,125, respectively). Similarly, mice immunised with SAS-adjuvanted *P. aeruginosa* AgN and subsequently challenged with acute *P. aeruginosa* pneumonia showed a significant reduction in bacterial lung colonisation (0.96-$\log_{10}$, $p=0.0268$) and robust total IgG, IgG1, and IgG2a antibody titres (1: 312,500, 1: 1,562,500, and 1: 12,500, respectively).

Th1 and Th17 cellular responses may contribute to protection against these pathogens. ELISpot analysis revealed that both *A. baumannii* AgN and *P. aeruginosa* AgN elicited substantial IFN-γ-responses (4-fold, $p=0.0035$ and 6.4-fold, $p=0.007$, respectively) and IL-17 responses (9.2-fold, $p<0.0001$ and 5.8-fold, $p=0.0286$, respectively) compared to the adjuvant-only control.

These findings highlight those novel antigen homologs as promising candidates for a multivalent vaccine against antibiotic-resistant *P. aeruginosa* and *A. baumannii.*

B304

Adaptation to Hypoxia Promotes Oxidative Stress Resistance in Chronic *Burkholderia cenocepacia* Infection

Ciarán Carey ORCID iD, Alanna Doherty, Joanna Drabinska ORCID iD, Niamh Duggan ORCID iD, Siobhán McClean ORCID iD

UCD, Dublin, Ireland

Abstract

The cystic fibrosis (CF) lung is notable for the presence of steep oxygen gradients. *B.cenocepacia* must adapt to this low oxygen niche in order to successfully colonise. *B.cenocepacia* can also survive and replicate inside macrophages, which is a hallmark of Bcc chronic infection. To investigate whether hypoxia drives adaptation of *B.cenocepacia* in the CF lung and consequently tolerance to oxidative stress within macrophages, an early CF clinical isolate was continually cultured in either hypoxia (6% oxygen) or normoxia for 22-days in-vitro.

We previously reported that a late clinical CF isolate showed an increased abundance of 149 proteins relative to the respective early isolate. A comparison of the proteomes of hypoxia-adapted cultures (HACs) revealed consistent changes in the abundance of 81 proteins associated with chronic infection, indicative of fixed adaptations. These include proteins implicated in resistance to oxidative stress: universal stress proteins, hmpA, ahpC and ahpD.

Consistent with the adaptations in late clinical isolates, the attachment of HACs to CFBE41o- cells increased by ~2-fold ($p<0.01$). Protease activity was significantly elevated in HACs relative to both normoxia-adapted cultures (NACs) and the early isolate ($p<0.0001$). The HACs also exhibited increased resistance to ceftazidime ($p<0.05$) and ciprofloxacin ($p<0.05$). Moreover, a concomitant 2-fold increase ($p<0.01$) in survival of the late isolate in PBMC-derived CF macrophages relative to the early isolate was shown, while HACs also showed 2-fold greater survival ($p<0.05$) in CF-macrophages relative to NACs. Ultimately, these data confirm that hypoxia plays a key role in the mechanism(s) underlying adaptation, including oxidative stress tolerance.

B305

Survival in the bloodstream modulated by Tca proteins.

Evie Blake[1], Ruth Massey[1,2], Samuel Fenn[1]

[1]School of Microbiology, University College Cork, and APC Microbiome Ireland, Cork, Ireland. [2]2 School of Cellular and Molecular Medicine, University of Bristol, Bristol, United Kingdom

Abstract

Staphylococcus aureus is the leading cause of sepsis, with over 300,000 deaths in 2019. The bloodstream is a heavily protected niche, where *S. aureus* must withstand many host antimicrobials to establish an infection and cause sepsis. TcaR, a predicted MarR-family transcriptional regulator, has previously been identified to regulate several virulence genes, associating it with adhesion and surface protein expression. As part of the teicoplanin associated locus (tca), TcaR was hypothesised to also regulate *tcaA*, which remodels the cell wall to adapt to the bloodstream environment. Transposon mutant strain, *tcaR::tn*, has significantly increased survival upon exposure to serum fatty acids, antimicrobial peptides, and hydrogen peroxide, when compared to wild type strain JE2, to a greater degree than seen with *tcaA::tn*. Similarly to *tcaA::tn*, *tcaR::tn* also has a less negatively charged cell wall compared to wild type, together suggesting that TcaR is involved in regulating *S. aureus* cell wall structure and changing its ability to survive in serum. Current investigations are into the wall teichoic acid and membrane composition of *tcaR::tn* mutant strains. Additionally, TcaR protein has been successfully purified, to enable further investigations into the DNA binding capabilities of this regulator, and its relation to *tcaA* expression.

B306

A multifunctional polymer coating for eradication of catheter-associated urinary tract infections

Shuai Zhang ORCID iD, Kaiyi Xu

Queen's University Belfast, Belfast, United Kingdom

Abstract

Catheter-associated urinary tract infections (CAUTIs) are among the most common hospital-acquired infections worldwide, affecting over 150 million people annually and placing substantial burdens on healthcare systems. The treatment of CAUTIs is becoming increasingly challenging due to the rise of antimicrobial-resistant (AMR) pathogens. While a few anti-infection catheters are in clinical use, no current technology can provide long-term (>30 days) infection control. The root cause lies in the complex pathogenesis of CAUTIs, which involves a cascade of events, including inflammation-induced host protein deposition, bacterial swarming migration, biofilm formation, and encrustation. Traditional strategies to combat CAUTIs focus on pathogen elimination or preventing attachment, but an ideal catheter addressing all pathogenesis-related factors remains undeveloped. In this research, we developed a range of coatings to target the entire pathogenesis process of CAUTIs, combining super-repellent polymer brushes (SPB), anti-swarming inhibitors (ASI), and antimicrobial nanozymes (ANZ). Results showed that SPB and ASI copolymerized at a 1:1.6 molar ratio retained a slippery, liquid-like coating that effectively inhibited trauma-induced inflammation, protein deposition, and *Proteus mirabilis* adhesion and migration. TiO_2-based ANZs demonstrated a two-fold reduction in MIC/MBC against *Proteus mirabilis* compared to nitrofurantoin and showed no AMR within 28 days. Coatings combining SPB, ASI, and ANZ at a 1:1.6:2.1 molar ratio exhibited potent antibiofilm activity, reducing biomass accumulation by over 99.9% after seven days compared to commercial antimicrobial catheters. This study presents a new design paradigm for next-generation anti-infection surfaces.

B307

3D microvascular co-culture models reveal the impact of dengue virus non-structural protein-1 on endothelial-pericyte dysfunction

Justin Duruanyanwu[1,2], Emma Brown[2,1], Fatima Abukunna[1], Paola Campagnolo[1], Kevin Maringer[2]

[1]University of Surrey, Guildford, United Kingdom. [2]The Pirbright Institute, Woking, United Kingdom

Abstract

Dengue virus (DENV) consists of four serotypes, with half of the world at risk of dengue infection. DENV infection can progress to severe dengue, characterised by extensive and diffused vascular dysfunction, leading to haemorrhage, organ failure and shock. This is initiated through microvascular permeability, though the mechanisms underlying this are not completely clear. Previous work showed DENV-2 Non-structural protein-1 (NS1) to disrupt interactions between endothelial and perivascular cells (pericytes), amplifying leakage. Here, we seek to further investigate this and elucidate the underlying mechanism.

Utilising a transendothelial electrical resistance (TEER) assay with human umbilical vein endothelial cells (HUVECs) and saphenous vein pericyte (SVP) co-cultures, NS1 from all four serotypes of DENV was shown to reduce pericyte's support of endothelial barrier function, increasing endothelial permeability. Furthermore, DENV NS1 reduced pericyte's ability to support 3D vascular structure formation in Geltrex. We developed novel 3D microvascular spheroids and found that NS1 treatment modified the organisation of these endothelial-pericyte spheroid co-cultures.

At a mechanistic level, DENV NS1 decreased the production of pro-angiogenic factors in pericytes by RT-qPCR of SVPs. Additionally, a proteomic secretome analysis identified a novel mediator of vascular permeability, which we produced recombinantly and found to reduce endothelial barrier function in a HUVEC TEER assay.

Overall, DENV NS1 was shown to disrupt endothelial-pericyte interactions, affecting their physiological function and inducing leakage. Furthermore, we identified a novel regulator of vascular permeability of relevance during DENV infection, and potentially for other haemorrhagic viruses. These findings pave the way for establishing future treatments and diagnostics.

B310

Three novel effective vaccine antigens protect against *Klebsiella pneumoniae* infection

Yueran Hou[1], Paulina Zarodkiewicz[2], Julen Tomás Cortázar[1], Nouran Naged[1], Dominic Stoner[1], Maryna Startseva[1], Miguel Valvano[2], Becky Ingram[2], Siobhán McClean[1]

[1]University College Dublin, Dublin, Ireland. [2]Queen's University Belfast, Belfast, United Kingdom

Abstract

Klebsiella pneumoniae is a major opportunistic pathogen, associated with high mortality rates. It was declared a priority pathogen by the WHO due to its worrying antimicrobial resistance, yet despite its devastating effects, there are no licenced vaccines against this pathogen.

We have identified proteins used by *K. pneumoniae* to attach to host epithelial cells and examined their potential as novel protective vaccine antigens. In total, 31 bacterial adhesins from *K. pneumoniae* were identified by the Cell-Blot proteomic approach, 24 of which were previously unidentifed. Three antigens were selected as potential vaccine candidates. These were confirmed to play a role in *K. pneumoniae* attachment to lung cells *in vitro*, as BL21 cells expressing recombinant proteins showed 14.3–, 7.22–, and 6.48–fold (p=0.012, p=0.0199, p=0.0011) increased attachment to human lung cells respectively. Immunisation of mice with Antigens LysM/BON, Dps or OMPP1 individually showed reduced *K. pneumoniae* peritoneal burden (1.54 log, 2.69 log, 2.50 log CFU (p=0.0080, p=0.0007, p=0.0007), respectively) in a sepsis challenge model and also reduced dissemination to the spleen (by up to 1.4 $\log_{10}$ CFU). Serological analysis showed the expression of high antigen–specific antibody responses compared to controls. T–cell recall response measured following immunisation of Antigens LysM/BON, Dps or OMPP1 showed significantly increased stimulation of IL-17 (5.11-, 4.81, and 4.79-fold (p=0.0071, p=0.0155, p=0.0197), respectively, relative to controls) while IL-4 responses were significantly increased following immunisation with Antigen OMPP1 (2.27-fold, p=0.0175). Overall, these antigens were highly protective against *K. pneumoniae* sepsis and have potential as vaccine candidates.

B311

Investigations into utility of a lipid A deficient *E. coli* strain in therapeutic bacteriophage production.

Emily Welham[1,2], Alison Low[1,2], Marianne Keith[1,2], Sean McAteer[1,2], David Gally[1,2]

[1]The University of Edinburgh, Edinburgh, United Kingdom. [2]The Roslin Institute, Edinburgh, United Kingdom

Abstract

Endotoxin reduced *E. coli*, ClearColi™, is used to produce recombinant proteins under commercial license. This strain or equivalent derivatives could prove useful in therapeutic bacteriophage (phage) production. The parental strain, BL21 (DE3), and LPS deficient ClearColi™ were compared. Initially, efficiency of phage infection was assessed using a phage panel (n=31) in liquid assay. While a similar host range was found, 21/31 infected BL21 (DE3) and 18/31 phage infected ClearColi™, the three phage that failed to infect ClearColi™ had a range of targets, including outer membrane protein C. A limiting factor in bacteriophage production is the presence or lack of a phage receptor. Through expanding receptor diversity, a strain can propagate a wider range of phage. Due to a gene mutation, BL21 and ClearColi™ do not express OmpC. It was demonstrated that after chromosomal restoration of the OmpC receptor, an OmpC-dependent phage could infect and be produced from the modified ClearColi™. Burst size and virion number were studied to explore the two strains' production capacity. While ClearColi™ grows slightly slower, with smaller bacteria, there was no significant difference in virions produced by equivalent optical densities of the two strains for the majority of infecting phage. Phage preparations from ClearColi™ confirmed a marked reduction in endotoxicity as assessed using a reporter cell line. This study demonstrated that an LPS modified, endotoxin-reduced, strain of *E. coli* has capacity to be a good therapeutic phage host. Phage therapeutics produced would demonstrate reduced toxicity and subsequently would have reduced post-production clean-up costs and improved safety.

B312

Modelling Insertion Sequence-Driven Evolution of Bacterial Genomes

Iain MacArthur ORCID iD

Northumbria University, Newcastle, United Kingdom

Abstract

Bacteria evolve faster than their hosts, making it imperative to understanding the mechanisms behind this to develop strategies for controlling bacterial pathogens. Mobile genetic elements, specifically insertion sequences, play a major role in remodelling bacterial genomes and driving evolution.

This study focuses on two genetically very similar species of the genus *Bordetella*: one, *Bordetella pertussis*, causes a severe disease in humans (whooping cough), whilst the second, *Bordetella bronchiseptica*, causes less severe disease but in a wide range of animals. All reported genomes of *B. pertussis* contain ~250 copies of IS481 but are absent from *B. bronchiseptica*, and therefore their role in species diversification must be important. *B. pertussis* has evolved from a *B. bronchiseptica* like ancestor and the DNA shared between these two species remains >99% identical. However, the genome of *B. pertussis* has undergone large numbers of rearrangements and deletions driven by the activity of IS481.

To investigate IS481's role, we introduced it into the *B. bronchiseptica* chromosome and sequenced 60 isolates with at least one IS481 copy to determine initial integration sites. Using the Chi.Bio continuous culture system, we have grown multiple lineages of *B. bronchiseptica* IS481 for over 1,000 generations, quantifying IS481 copy numbers, and assessed the phenotypic changes caused by IS481 genome rearrangement.

This model allows us to examine bacterial genome remodelling mechanisms mediated by insertion sequences, offering new insights into the evolutionary processes underlying *B. pertussis* pathogenesis and broader bacterial genome dynamics. Such understanding is essential to develop predictive models for bacterial evolutionary potential.

B313

First antigenic exposures to SARS-CoV-2 Spike do not indelibly shape SARS-CoV-2 immunity

David Greenwood[1], Joshua Gahir[1], Marianne Shawe-Taylor[1], Hermaleigh Townsley[1], Crick COVID Surveillance Unit[1], Crick COVID Serology Pipeline[1], Legacy Investigators[1,2], Mary Y Wu[1], Edward J Carr[1], David LV Bauer ORCID iD[1], Emma C Wall[1,2]

[1]The Francis Crick Institute, London, United Kingdom. [2]NIHR University College London Hospital BRC, London, United Kingdom

Abstract

Vaccination has been extraordinarily effective against severe COVID-19, but crucial questions remain unanswered, including how first exposure impacts long-term immunity and infection risk.

We investigated infection risk and antibody-mediated immunity in participants of the UCLH-Crick *Legacy* study, which has followed participants since early 2021. The Legacy study is unique since it incorporates weekly occupational health testing data for SARS-CoV-2, along with longitudinal serological analysis.

Participants were grouped by first exposures to SARS-CoV-2 spike (infection-first, n=54, vs. vaccination-only, n=177). We found that while infection-first individuals exhibited higher nAbT to all tested variants before and after dose 2, those who were initially in the vaccination-only rapidly increased their neutralising antibody titres against all variants over the course of 2022, with no sign of an indelible mark of “original antigenic sin” between these two groups.

Despite slight differences in neutralising antibody titres, however, the infection-first cohort had a reduced risk of infection beyond 300 days, during the Omicron BA.2/BA.5 waves in 2022, compared to the vaccination-only cohort ($p<0.001$), suggesting that further mechanisms of protection beyond serum neutralising antibodies, such as mucosal and cellular immunity, continue to offer an element of protection against emerging SARS-CoV-2 variants.

B314

Antimicrobial resistance and pathogen translocation from the gut in haematopoietic stem cell transplant recipients in India

Archana Madhav ORCID iD[1,2], Jobin John Jacob[3], Sushil Selvarajan[4], Sanika Kulkarni[3], Yesudoss M[3], Sourav Das[4], Plamena Naydenova[1], Biju George[4], Balaji Veeraraghavan[3], Stephen Baker[1,2]

[1]Cambridge Institute of Therapeutic Immunology and Infectious Diseases, University of Cambridge, Cambridge, United Kingdom. [2]Wellcome Sanger Institute, Hinxton, United Kingdom. [3]Department of Clinical Microbiology, Christian Medical College, Vellore, India. [4]Department of Haematology, Christian Medical College, Vellore, India

Abstract

Haematopoietic stem cell transplant (HSCT) patients present a challenge in clinical settings as their gut environments harbour numerous conditions that favour pathobiont colonisation. These include the use of broad-spectrum antimicrobials which deplete the commensal microbiota, mucosal barrier injury (MBI) as a side effect of antacids administered to counter chemotherapy-induced dyspepsia and altered ecology of bowel microbiota triggered by pre-transplant conditioning regimens. In the pre-engraftment period, HSCT patients are highly susceptible to developing febrile neutropenia and sepsis, with the gut frequently implicated as the primary source of pathobiont translocation into the bloodstream following MBI. Despite infection prophylaxis attempts with intestinal decontamination, antimicrobial-resistant enteric pathogens have proven resilient and frequently cause invasive antimicrobial resistant (AMR) infections post-transplantation. Here, we present the ARBOMAT study, where we applied enrichment-based metagenomic sequencing on 252 longitudinally collected stool samples from 81 HSCT patients in India to select for common AMR-carrying bacteria that cause bloodstream infections (BSI). Our sampling strategy allowed us to monitor longitudinal fluctuations in AMR gene diversity within HSCT patients' gut resistomes and enabled us to reconstruct 'high quality' (>90% completeness; <5% contamination) metagenome-assembled genomes to track the spatiotemporal movement of shared sequence types (99.999% nucleotide identity). We also found five BSI episodes where pathogenic bacteria had likely translocated from the GI tract. To our knowledge, this is the largest study characterising the gut resistomes of HSCT patients conducted in a lower-middle income country to date, using genomics to explore gastrointestinal AMR carriage and bacterial translocation among high-risk HSCT patients in this region.

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