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#Microbio25

INVITED AND OFFERED TALKS

MICROBIOLOGY SOCIETY

Contents

AMR – Mechanisms & Regulation

Invited: Regulation of Mycobacterial cell wall metabolism contributes to antibiotic resistance and tolerance

Cara Boutte ORCID iD, Neda Habibi Arejan, Karen Tembiwa, Manuel Chavez

UT Arlington, Arlington, USA

Abstract

Mycobacterial cell walls are distinctive in two ways: first, they have an unusual covalently-linked outer membrane; and secondly, cell wall expansion occurs at the poles, rather than the lateral walls, of these rod-shaped bacteria. The importance of the mycolic-acid outer membrane in antibiotic resistance is well known, as this structure is both an important permeability barrier and antibiotic target. Our recent work indicates that the system of polar growth also has implications for antibiotic treatment. We have found that Wag31, the DivIVA ortholog that helps recruit cell wall proteins to the pole, is also involved in controlling their dissociation from the pole during stress. We have shown that MmpL3, the transporter of mycolic acid precursors and an important drug target, is localized through an interaction with Wag31, and that this interaction domain is important both for polar localization, and for regulation of cell wall metabolism and antibiotic resistance. We are working to determine how the dynamic localization of essential mycolate-binding protein PgfA and peptidoglycan enzyme PonA1 affect both the regulation of cell wall metabolism and tolerance to antibiotics under stress.

Invited: Transmission and transmission inhibition of AMR plasmids in *E. coli* and *K. pneumoniae*

Michelle Buckner

University of Birmingham, Birmingham, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a growing problem, especially in Gram-negative bacteria such as *Klebsiella pneumoniae* and *Escherichia coli.* Many AMR genes are carried on mobile genetic elements, including plasmids, which mediate horizontal gene transfer between bacteria. Such conjugative plasmids contribute to AMR gene dissemination within and between bacterial species. Because of the important role that plasmids play in AMR dissemination, we have performed high-throughput screens to identify potential "anti-plasmid" compounds, which could be used to reduce plasmid prevalence in targeted bacterial communities. We have identified a group of FDA approved drugs, the nucleoside analogues, as putative anti-plasmid compounds. Our current work is focusing on characterising the mechanism of action by which these drugs inhibit conjugation. In addition to nucleoside analogues, we have also been exploring natural products and novel cobalt-based compounds with some anti-plasmid activity. We use a combination of different plasmids in *E. coli* and *K. pneumoniae*, including clinical isolates/plasmids to assess compound efficacy. In the long term, such anti-plasmid compounds could be a useful scientific tool for understanding plasmid biology, or even a targeted treatment to remove plasmids from bacterial populations.

Invited: Ecological and evolutionary mechanisms of antibiotic resistance emergence within patients

Brockhurst Michael ORCID iD

University of Manchester, Manchester, United Kingdom

Abstract

Clinical antibiotic treatments can select for resistance within treated patients, however the ecological and evolutionary mechanisms of within-patient resistance emergence and their relative importance are unclear. Clinical trials for antibiotics provide a powerful tool to quantitatively study resistance emergence due to well-defined dosing, regular sampling and placebo controls. Using high-throughput phenotyping and genome sequencing of >30,000 *Pseudomonas aeruginosa* clinical isolates sampled from 180 bronchiectasis patients over 1 year, we show that resistance emerges through a variety of mechanisms. These include pre-existing resistance, de novo mutation, and strain replacement, and, in multiple cases, complex combinations of these mechanisms. We explore the predictability of resistance emergence and how this varies by mechanism, and what implications this has for improving diagnosis and clinical treatments.

Invited: Investigations into the intracellular mobility of AMR genes and associated mobile genetic elements.

Adam Roberts ORCID iD, Richard Goodman ORCID iD

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Abstract

Horizontal gene transfer of antimicrobial resistance (AMR) genes is a well studied phenomenon and is recognized as an important mechanism for the dissemination of AMR genes between bacteria. What is less well studied is AMR gene mobility within bacteria cells, despite its importance for example in co-localizing multiple AMR genes on the same mobile genetic element (MGE). We have recently developed and validated a MGE entrapment plasmid which enables us to positively select for rare transposition and recombination events between replicons (chromosomes and plasmids) within *Escherichia coli* populations. In this presentation we will outline the functionality of the entrapment system and describe the results of multiple assays we have developed to investigate different aspects of intracellular mobility including response to antimicrobial agents. We detected multiple transposition events of a myriad of MGEs and AMR genes and were able to detect rarer events such as chromosomal integration of plasmids, plasmid recombination, gene amplification and how transposition activity is affected by different concentrations of antibiotics. This entrapment plasmid provides a robust and reproducible experimental system to investigate mobility of AMR genes on a sub-cellular scale.

Experimental evolution of antibiotic resistance in standard and host-mimicking media

Charlotte Cornbill[1], Alasdair Hubbard[2], Freya Harrison[1]

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

Abstract

Understanding the evolution of AMR is integral to limiting AMR development and spread. Most laboratory AMR evolution experiments use standard laboratory media (e.g. cation-adjusted Mueller Hinton broth (caMHB)), but the environment can influence AMR evolution due to differing selection pressures and fitness costs. Here, we used experimental evolution to identify any differences in the adaptive landscapes of meropenem resistance in caMHB and two different host mimicking media.

Pseudomonas aeruginosa was selected for resistance to meropenem in caMHB, synthetic wound fluid (SWF) and synthetic cystic fibrosis sputum (SCFM) using an evolutionary ramp approach. The fitness of evolved clones was then assessed through antimicrobial susceptibility testing, growth curves and biofilm formation assays in a Calgary device.

The host mimicking media caused higher level resistance to meropenem, collateral resistance to cetriazone and levofloxacin, and increased susceptibility to amikacin in comparison to caMHB . Diverse fitness affects were observed depending on the media, in which they were selected. SWF evolved clones were able to produce 5-fold more biofilm than caMHB and SCFM evolved clones. Comparative fitness identified a reduction in area under the curve for caMHB-evolved clones compared to the ancestor which were not present in clones evolved in host mimicking media. Causal and compensatory mutations were identified using whole genome sequencing.

The choice of media caused differences in resistance profiles and fitness costs. Future antimicrobial testing should take this into consideration as part of any translational work to move candidate drugs to the clinic and to better optimise our current antimicrobials in the clinic.

Expanding UniProt annotation coverage for antimicrobial resistance

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EMBL-EBI, Cambridge, United Kingdom

Abstract

The UniProt Knowledgebase is a leading resource of protein information, providing the research community with a comprehensive, high quality and freely accessible platform of protein sequences and functional information. Manual curation of a protein entry includes sequence analysis, the expert summation of functional information from literature into relevant entries, and the identification of orthologs. This work facilitates scientific discovery by organising biological knowledge into machine- and human-readable data, enabling researchers to rapidly comprehend complex areas of biology.

Given the urgent public health threat of AMR, UniProt has initiated a project to improve annotation of proteins from key organisms prone to developing AMR. We have focused on the manual curation of various protein classes with a known role in AMR, including beta-lactamases, efflux pumps and ABC transporters. We aim to broaden the scope of the pipelines which transfer information, such as protein names, functions, catalytic activities, keywords, and Gene Ontology terms, from expert-curated protein entries from well-studied organisms onto orthologous proteins in related organisms. This may be via rule sets or by machine learning.

UniProt annotations are widely disseminated to other databases, including Rhea, EnsemblBacteria, and PATRIC (Bacterial & Viral Bioinformatics Resource Center) and we aim to extend links to AMR-focused resources like the Comprehensive Antibiotic Resistance Database (CARD).

Over 120 AMR-related proteins have been manually curated, and many previously lacked such annotations. We welcome suggestions for further curation targets to enhance support for ongoing AMR research.

Correlating phenotypic and genotypic determinants of biocide tolerance and adaptation in *Proteus mirabilis*

Vicky Bennett[1], Ocean Clarke[2], Maryam Ravari[1], Matthew Wand[3], Emma Denham[1], Mark Sutton[3], Brian Jones[1]

[1]University of Bath, Bath, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]UKHSA, Porton Down, United Kingdom

Abstract

Using a combined approach of adaptive evolution studies, whole genome sequencing, and phenotypic analysis, we have explored mechanisms underlying biocide tolerance in 78 *Proteus mirabilis* clinical isolates. This pathogen is a common cause of catheter-associated urinary tract infections, and often exhibits high tolerance to chlorhexidine (CHD), a biocide widely used in clinical infection control procedures. We previously identified that upregulation of the SmvA efflux system and alterations in lipopolysaccharide structure are key to this tolerance. However, the prevalence of these mechanisms and their association with tolerance to other biocides is unclear.

Genome analysis showed truncations in the *smvR* repressor and *mipA* genes, resulting in SmvA efflux upregulation and alterations in membrane permeability, are highly prevalent among clinical isolates. These mutations are significantly associated with high tolerance to multiple biocides, and frequently emerge in CHD adapted populations. Additionally, exposure to concentrations of CHD as low as 8µg/mL resulted in adaptation to the highest tolerance levels of CHD (>512µg/mL) through the same mutations, as well as cross tolerance to other biocides.

While these mechanisms facilitate decreased biocide susceptibility, they generally would not protect against biocides at clinically used concentrations. However, prolonged exposure to suboptimal biocide concentrations could select for increasingly tolerant strains, promoting the emergence of heterogeneous subpopulations. These subpopulations could have a selective advantage under more challenging real-world conditions where poor compliance with recommended biocide usage may occur.

Overall, we highlight the prevalence of mutations driving biocide tolerance, and the potential for adaptive mutations to challenge infection control efforts.

Proteomic and metabolomic responses of the Gram-positive bacteria *Enterococcus faecium* NCTC13169 to sub-inhibitory concentration of chloramphenicol

Monika Subanovic ORCID iD, Dean Frawley ORCID iD, Ciara Tierney ORCID iD, Trinidad Velasco-Torrijos ORCID iD, Fiona Walsh ORCID iD

Maynooth University, Maynooth, Ireland

Abstract

Enterococcus faecium is a high priority human pathogen associated with hospital-acquired nosocomial infections and prone to antimicrobial resistance. Chloramphenicol inhibits protein synthesis but, at sub-inhibitory concentrations (sub-MIC) may influence a cascade of cellular pathways affecting bacterial physiology beyond growth defects. Using integrated untargeted metabolomic and proteomic analysis, we investigated the effects of sub-MIC levels of chloramphenicol on a susceptible *E. faecium* NCTC13169. Functional enrichment analyses of differentially expressed proteins (DEPs) revealed that gene ontology terms associated with protein synthesis and ribosome assembly were the most enriched with up-regulated proteins. Protein-protein interaction analysis highlighted a major sub-network related to translation and ribosome assembly connected with sub-networks related to nucleotide metabolism, cell cycle and peptidoglycan biosynthesis, protein repair and oxidative stress response. Chloramphenicol treatment impaired oxidative stress management, indicated by the down-regulation of key transcriptional regulators (SpxA and MarR) and key chaperones suggesting disruptions in redox balance and protein folding and repair. Increased and decreased abundance of respective glutathione and hypoxanthine suggest a link with oxidative stress and biofilm formation management. Alterations in LuxS and DivIVA abundance indicate disruption in biofilm formation pathways. Additionally, sub-MIC levels of chloramphenicol affected cell cycle and peptidoglycan biosynthesis machinery, altering divisome complex-associated proteins. Metabolomic-proteomic results revealed perturbations in the regulation of central nitrogen and carbon metabolism. For the first time, we report trimethylamine metabolism altered by chloramphenicol. Overall, our findings suggest that sub-MIC chloramphenicol disrupts redox homeostasis, biofilm formation, survival and cell division molecular pathways in *E. faecium* NCTC13169, shedding light on its multifaceted cellular impacts.

Within-patient evolution of ciprofloxacin resistance in *Pseudomonas aeruginosa* across a large-scale clinical trial

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

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Abstract

The antimicrobial resistance (AMR) crisis threatens to endanger modern medicine within the next 20-30 years. A key part of the problem is the emergence of AMR within patients themselves, during courses of antibiotic therapy. Whilst within-patient AMR emergence is increasingly receiving attention in the literature, investigations commonly involve a small number of patients and study single-patient cases which may poorly represent the more general patient population. Here we report finding on the evolution of ciprofloxacin resistance by *Pseudomonas aeruginosa* across a large-scale clinical trial. This trial utilised inhaled liposomal ciprofloxacin as therapy for patients with the lung condition bronchiectasis, who suffered with *P. aeruginosa* infection. We isolated a total of ~25,000 independent bacterial colonies, prior to treatment and during the year-long the trial, measured the ciprofloxacin MIC and growth rates in KB broth for all isolates, and performed whole genome sequencing of ~4069 isolates. Across the trial, ciprofloxacin MICs increased during periods of treatment and decreased during treatment withdrawal, in a pattern suggestive of resistance fitness trade-offs operating within patients. We also find that distinct phenotypic adaptive trajectories are followed in different patient cases, indicating diverse evolutionary dynamics driving resistance emergence. We also characterise the genetic mechanisms driving the within-patient evolution of resistance and highlight that this occurs through at least 3 distinct mechanisms for *P. aeruginosa.* These findings allow us to start characterising the mechanisms through with AMR can emerge within-patients during treatment, and to ask questions on how best we could predict resistance emergence in the future.

Genome-Wide Analysis of Innate Susceptibility Mechanisms in *Escherichia coli* Under Ciprofloxacin Stress

Muhammad Yasir ORCID iD[1,2], Keith Turner[1,2], Sarah Bastkowski[1,2], Andrea Telatin[1,2], Ian Charles[1,2,3], Mark Webber[1,2,3]

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Abstract

Ciprofloxacin, an essential antibiotic for treating multidrug-resistant (MDR) bacterial infections, exerts its bactericidal effect primarily through inhibition of topoisomerase genes and target site mutation is a major mechanism of resistance. However, many other genes have been implicated in determining ciprofloxacin susceptibility. To explore this complexity, we conducted a genome-wide screen using Transposon Directed Insertion-site Sequencing with expression (TraDIS-Xpress) in the model organism *Escherichia coli* BW25113. This approach enabled comprehensive analysis of the genome, including essential genes, to uncover previously unrecognized genetic determinants of ciprofloxacin susceptibility.

Our findings confirmed several known resistance pathways including key roles for DNA repair genes including *recA*, *polA*, *priA*, *seqA*, *dinG*, *recN*, *recABC*, and *dnaG*. Furthermore, regulatory genes including *marR*, *iscR*, *acrR*, *clpS*, *ohsC*, *oxyS*, *soxR*, and *uspC* were implicated in modulating susceptibility. Enhanced sensitivity to ciprofloxacin was observed with the disruption of efflux systems and surface-associated functions, while mutations that conferred decreased susceptibility were predominantly associated with genes involved in surface polysaccharide biosynthesis. Our data also identified potential novel roles for genes including *hfq*, *clpS* and *sspA* in ciprofloxacin susceptibility.

Complementing the genome-wide analysis, microfluidics experiments were conducted to investigate the behaviour of individual mutants within droplet-based communities under ciprofloxacin stress. Overall, our study broadens the understanding of fluoroquinolone impacts on bacterial cell biology and metabolism, offering new perspectives on the wider range of mechanisms bacteria employ to withstand antibiotic pressure.

Metabolism triggers phenotypic resistance in Mycobacterium tuberculosis

Jordan Pascoe[1], Mark Laws[2], Jane Newcombe[1], George Mayson[1], Thomas Mendum[1], Gerald Larrouy-Maumus[3], Miraz Rahman[2], Dany Beste[1]

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Abstract

There is an urgent need for new approaches to treat tuberculosis (TB), however antibiotic development is exceptionally challenging due to the remarkable ability of the causative agent *Mycobacterium tuberculosis* to tolerate a variety of host and antimicrobial stressors. Recognizing the key role of bacterial metabolic state on antibiotic susceptibility; our research posits that the direct modulation of cell metabolism, using metabolites from specific pathways, has the potential to improve our understanding of how antibiotics exert activity and allow for the intelligent design of therapeutic interventions. Through this approach we have identified a metabolite that surprisingly triggers the rapid emergence of phenotypic resistance in *M. tuberculosis*. Exploring this phenotype using ^{13}C isotopomer analysis we have identified key pathways involved in this phenomenon. This work underscores a sophisticated metabolic strategy to mitigate collateral cellular stress to avoid antibiotic killing. Informed by these discoveries, we adopted a medicinal chemistry approach to synthesize adjuvant compounds to counter the development of resistance and potentiate antibiotic killing. Our research furthers the understanding of the bacterial stress response to antibiotic treatment and provides an innovative approach for development of more effective treatment strategies against one of the most successful pathogens on the planet.

Attacking the ribosomal machinery: Identification of Ribosomal Protein Targets for Novel Antibiotic Development in Antimicrobial-Resistant Pathogens

Priyanshu Raikwar, Martin Maiden, Samuel Sheppard

University of Oxford, Oxford, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a known global challenge. Ribosomal proteins, essential , but less studies components of the bacterial protein synthesis machinery, which can be plausible drug targets. However, there is a need for a more systematic approach to identifying new ribosomal targets. We explore the variability and conservation among ribosomal proteins across diverse bacterial species, aiming to identify those that can serve as robust targets for next-generation antibiotics. We employed a suite of biostatistical methods with GWAS studies. to assess the evolutionary pressures acting on 53 ribosomal protein subunits (*rps* genes). This approach helped us to isolate plausible targets that are both conserved and essential for bacterial viability, while also pinpointing proteins that may be associated with resistance mechanisms. To further refine our target selection, we conducted masked and conditional genome-wide association study (GWAS) to identify resistant isolates and their corresponding binding pockets. The identified targets are then subjected to a fragment-based drug discovery approach, integrating pharmacophore modelling, chemical binding assays, and pharmacokinetic studies to develop potential inhibitors.

From the hits we get, we aim to perform *in-vitro* studies, specifically focussing on ESKAPE pathogens. Despite the scarcity of current efforts to target ribosomal proteins, our research proposes a straightforward yet comprehensive strategy to overcome the challenges posed by AMR, paving the way for innovative therapeutic solutions.

Genetic characterisation of dominant NDM-encoding plasmids in North-West London in late 2023: A Hybrid-assembly Approach

Rojus Urbonas[1], Elita Jauneikaite[1], Martina Chukwu[1], Jonah Rodgus[1], Maria Getino[1], Yu Wan[1,2], Frances Davies[1,3]

[1]NIHR Health Protection Research Unit in Healthcare Associated Infections and Antimicrobial Resistance, Department of Infectious Disease, Imperial College London, Hammersmith Hospital, London, United Kingdom. [2]David Price Evans Global Health and Infectious Diseases Research Group, Department of Pharmacology and Therapeutics, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, United Kingdom. [3]Imperial College Healthcare NHS Trust, London, United Kingdom

Abstract

Carbapenems are last-resort antibiotics for treating multidrug-resistant (MDR) *Enterobacterales* infections, yet resistance to them is emerging. A key resistance determinant is the New Delhi metallo-beta-lactamase (NDM) gene, often carried on mobile genetic elements including plasmids, facilitating horizontal transfer between *Enterobacterales* species and posing a significant threat to vulnerable patients within healthcare settings.

Eight NDM-positive isolates were selected to investigate the genetic context of bla_{NDM} genes. These isolates were from a collection of 354 carbapenemase-producing Enterobacterales (CPE) from patients across North-West London between October 2023 and January 2024. These eight isolates underwent Oxford Nanopore MinION long-read whole-genome sequencing (WGS), complemented by Illumina WGS reads to obtain hybrid assemblies.

We sequenced three *K. pneumoniae* isolates (ST11, two ST147), three *E. coli* isolates (ST405, ST10, ST6662), one Citrobacter and *Enterobacter hormaechei* isolate, respectively. Each isolate had 2-4 plasmids, with only *K. pneumoniae* ST11 having 6 plasmids. Two isolates (*K. pneumoniae* ST11 and *E. coli* ST405) carried bla_{NDM-5} on *IncFIB/HI1B* and *IncF* plasmids, respectively; and the other isolates carried bla_{NDM-1} on *IncF*, *IncHI* or *IncFIB/HI* plasmids. All plasmids carried genes conferring resistance to at least two other antibiotic classes such as aminoglycosides and sulphonamides.

Detailed characterisation of MDR plasmids commonly found in CPE uncovers how such plasmids spread between bacterial species. This study has generated reference sequences of bla_{NDM}-carrying plasmids for further epidemiological and evolutionary investigations into this type of plasmid.

BSP/Protistology-UK: Tackling emerging eukaryotic microbial pathogen threats

Invited: Understanding host cell tropism in Zoonotic malaria

Melissa Hart, Amy Ibrahim, Franziska Mohring, Sophia DonVito, Morven Law, Kostas Kousis, James Thomas, Robert Moon ORCID iD

London School of Hygiene & Tropical Medicine, London, United Kingdom

Abstract

Whilst most malaria parasites exclusively infect humans, including *Plasmodium falciparum* the most significant cause of malaria deaths globally, *Plasmodium knowlesi* is an emerging cause of zoonotic malaria in SE Asia. The parasite naturally infects macaques but increasingly also causes infections in humans – including in areas that have otherwise eliminated all other malaria, like Malaysia. Understanding the determinants of host cell tropism – and why some parasites are able to skip species boundaries, is critical not only in developing control methods, but also in understanding the underlying biology of all malaria parasite species.

Malaria parasites rely on cycles of cellular invasion and intracellular growth to proliferate within the blood stream, a process which underpins symptoms of the disease, defines host cell tropism, and a potential target for vaccines. The proteins responsible for binding erythrocytes have been studied extensively in P. falciparum: the erythrocyte binding-like proteins and the reticulocyte binding-like proteins. These proteins are hypothesized to have overlapping but critical roles during the invasion process. *P. knowlesi*, has a smaller repertoire of these proteins, and much larger and polarised invasive stages known as merozoites.

Using CRISPR-Cas9 genome editing, we've demonstrated that whilst there are redundant pathways for invasion of macaque erythrocytes, a distinct subset are essential for invasion of human erythrocytes and therefore underpin zoonotic infection. The two families play distinct roles at different invasion stages. We also demonstrate that *P. knowlesi* merozoites undergo extensive gliding motility, which may provide a mechanism for cell-selection in non-ideal hosts, potentially underpinning their broad host range.

Invited: Awareness, drug discovery and diagnostic tools against pathogenic free-living amoebae.

Christopher Rice ORCID iD

Purdue University, West Lafayette, USA

Abstract

Pathogenic free-living amoebae (*Acanthamoeba* species, *Balamuthia mandrillaris,* and *Naegleria fowleri*) cause several neglected human diseases. Due to the difficulties in awareness and treating these diseases, the unmet clinical need is for highly potent, quickly acting therapeutics, and rapid diagnostics since misdiagnosis is common. An additional need is for potent cysticidal/recrudescence inhibitors for diseases caused by *Acanthamoeba* and *Balamuthia*. We developed novel high-throughput screening methods to screen various drug libraries in search for new active chemical scaffolds against pathogenic FLA. Herein, we report our efforts for screening the world's largest known number of drugs to identify potentially new anti-amoebic therapeutics. Lastly, we will provide an update on new highly sensitive and specific diagnostic tools developed for *Balamuthia mandrillaris* diseases. This is by far the largest screen of drugs for these neglected amoebae and these data identify new repurposing drug candidates for the treatment of amoebic diseases. By developing rapid diagnostics hand-in-hand we hope this will eventually help bring down the mortality of patients afflicted from amoebic diseases.

Invited: Responses of parasitic protists and their macroinvertebrate hosts to stressor exposure in aquatic and terrestrial habitats

Sonja Rückert ORCID iD

University of Duisburg-Essen, Essen, Germany

Abstract

TBC

Invited: Foodborne toxoplasmosis: investigating the role of retail meat

Jackie Thomson[1], Jacqueline Plaza[1], Filip Dámek[1], Isabelle Villena[2], Maike Jores[3], Gereon Schares[3], Elisabeth Innes[1], Frank Katzer[1], Clare Hamilton[1]

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Abstract

Consumption of infected, undercooked meat is a well-known risk factor for transmission of *Toxoplasma gondii*, and when comparing foodborne pathogens, *Toxoplasma* has been ranked as one of the most significant causes of disease burden both in Europe and the USA. Despite this, there have been few studies assessing the risk of infection from retail meat samples. The aim of this study was to investigate the presence of *T. gondii* in commercially available meat cuts, and to assess parasite viability in higher risk meats. Initially, 300 meat samples (beef, chicken, lamb, pork and venison) were purchased from retail outlets, and screened for *T. gondii* using quantitative PCR. Results revealed *T. gondii* DNA was present in 35.4% venison samples, 6.9% lamb samples, 4.8% chicken samples, 4.2% pork samples and 0% beef samples. Partial PCR-RFLP genotyping revealed non-clonal genotypes. Given the high incidence of *T. gondii* in venison and the propensity to consume this meat undercooked, parasite viability was determined in this meat type. Twenty-three fresh venison products were purchased, and *T. gondii* DNA was detected in 5 samples (21.7%). For each of the positive samples, 6 outbred CD-1 mice were inoculated and monitored for 28 days. Viable *T. gondii* was detected in 2 venison products. Tachyzoites were isolated and cultured from the positive mice, and PCR-RFLP analysis revealed a Type II-variant (#3) in both samples. These results highlight the potentially important role of game meat, specifically venison, in foodborne transmission of *T. gondii*.

A genetic screen in Candida albicans for modulators of the neutrophil oxidative burst

Huan Jiang, Borko Amulic, Angela Nobbs

University of Bristol, Bristol, United Kingdom

Abstract

Candida albicans (*C. albicans*) is an opportunistic fungal pathogen that poses serious threats to human health, especially in individuals with compromised immune systems. Neutrophils, as frontline immune responders, play a crucial role in controlling *C. albicans* infections through mechanisms such as oxidative burst, phagocytosis, degranulation and NETosis. However, *C. albicans* has evolved sophisticated immune evasion mechanisms to resist neutrophil-mediated killing, many of which remain uncharacterised. This project conducts a screen of the *C. albicans* GRACE (Gene Replacement and Conditional Expression) mutants library, using ROS production by human neutrophils as a readout. Our goal is to identify *C. albicans* factors that modulate the neutrophil response, either by (1) enabling detection by neutrophils or (2) evading neutrophil-mediated activation. We screening 700 mutants (30% of the GRACE mutant library), and identified 14 potential hits, 5 of which mutants were selected for further investigation. By exploring the genetic basis of *C. albicans* resistance to neutrophils, our study will contributes to a better understanding of fungal pathogenesis and reveal pathways to enhance host immune efficacy. *C. albicans* proteins that modulate neutrophils' antifungal response represent potential therapeutic targets for this growing global threat.

Under the Lens: Understanding drug resistance in *Acanthamoeba*

Ronnie Mooney, Fiona Henriquez

University of Strathclyde, Glasgow, United Kingdom

Abstract

The sight-threatening infection, *Acanthamoeba* keratitis, is normally associated with contaminated contact lens wear as the lenses can promote small tears in the corneal surface allowing entry of the pathogen. At present, disease treatment or prevention is difficult due to limited therapeutic options, with most current treatments being toxic to corneal cells and capable of inducing the transformation of *Acanthamoeba* active trophozoites into a highly resilient dormant cyst. Additionally, the susceptibility of *Acanthamoeba* to frontline treatments varies significantly between isolates, yet very little is known about their resistance potential, or the mechanisms that might support this. We have developed drug-resistant *Acanthamoeba* cell lines *in vitro* and isolated several from various anthropogenically polluted sources globally with the aim of understanding how resistance develops and how we can circumvent it clinically. The emergence of antimicrobial resistance (AMR) is a significant global threat and our data offers valuable insight to how AMR develops in *Acanthamoeba*. Here, we present data from several studies aimed at highlighting the extent of AMR-*Acanthamoeba* in the environment, and elucidating the mechanistic routes used by the protist to develop drug resistance. The results from this study will enable eye-care and pharmaceutical industries to remain one step ahead in the clinical arms race against *Acanthamoeba*.

Beyond the lab – Turning your research into reality

Update from the Department of Science, Innovation and Technology

Isabel Webb

Department for Science, Innovation and Technology, London, United Kingdom

Abstract

The Department for Science, Innovation and Technology is the UK Government department responsible for research and development, digital and emerging technologies. This includes policy to support engineering biology, identified in 2022 as one of the critical technologies for UK growth and resilience.

The UK's approach for engineering biology is set out in the National Vision for Engineering Biology. This document provides a roadmap for supporting the growth of this technology in the UK, including world leading R&D, infrastructure, skills and talent, regulatory environment and economic growth ecosystem.

Since the publication of the National Vision for Engineering Biology in December 2023 government has taken action to support growth of the technology through activities such as £100 million of UKRI investment into mission-led activity, and investment into novel regulatory approaches. DSIT, working closely with relevant partners from across government and the public sector, continues to develop and implement policy to support the growth of engineering biology and capitalise on the economic and societal benefits that the technology will bring.

This talk will provide an update on the implementation of the National Vision for Engineering Biology and wider work on emerging technologies and R&D within the UK.

Research to *real* impact through IP

Sara Holland

Potter Clarkson, Nottingham, United Kingdom

Abstract

Academics are well used to the term “impact” – mostly in the context of the “impact factor” of a journal. The higher the better, and the better for your academic career. But what does it take to have REAL impact – out there, beyond the academic system. Does the outside world care if you publish in Nature? Or does it care about real, tangible products and services that can benefit the world?

This is why we all went into science, **right**?

So how do you bring the vague idea you might have had at the lab bench to the world?

You do this through intellectual property – capturing and using those clever ideas you have come up with to either build your own business around, or licence to existing companies.

We will cover some key points I want all academics to know about intellectual property, and patents in particular.

How and Why to get Involved in Policy

Daniel Robinson, Esme Heppenstall-Harris

Economic and Social Research Council, Swindon, United Kingdom

Abstract

As a funder, we recognise the value of fostering stronger links between academia and policy to address society's most pressing challenges. Programmes like the UKRI Policy Fellowships are essential in embedding researchers directly into government, devolved administrations, and key research organisations, ensuring that high-quality academic insights shape public policy and its implementation. By seconding researchers into the heart of government, these fellowships enhance the flow of evidence and expertise, strengthening decision-making processes with rigorous, evidence-based research..

The Policy Fellowships, alongside initiatives such as the Thematic Research Leads and the Local Policy Innovation Partnerships, create meaningful opportunities for academics to engage with policymakers. These collaborations foster sustainable relationships and allow for real-time knowledge exchange, ensuring that research is not only accessible but actionable. Academics contribute critical, long-term perspectives and robust methodologies that often complement the more immediate demands of policymaking. Collaboration between academia and policymakers can lead to more effective, sustainable policies grounded in data and sound research. By supporting academic involvement in policy, we can ensure that research translates into tangible, real-world solutions.

By supporting these engagements, we aim to build a strong, evidence-based foundation for policy across sectors ensuring that research continues to have a lasting and positive influence on public policy, public services, and societal well-being.

Building partnerships between industry and researchers to tackle the 'problems worth solving by us'

Matthew Gilmour ORCID iD

Quadram Institute, Norwich, United Kingdom

Abstract

UK consumers expect their food to be safe, and food producers and government are vigilant and work diligently to ensure safety of food supplies. The Food Standards Agency reports that food-associated incidents are most commonly due to pathogenic microorganisms and that challenges remain to fully understanding and controlling pathogen entry into the food chain. Many factors beyond 'food microbiology' are needed to understand microbial risks, and it is essential that research on innovative food safety solutions is transdisciplinary, bringing food producers and government departments together with researchers with expertise across a range of scientific disciplines to link and amplify their specialisations. In this session we will discuss how these researchers and government departments are partnering with food businesses that share a forward-thinking vision about the nature of microbial risks and how they can be controlled with new scientific innovations, behaviours and actions. Through collaboratives such as the Food Safety Research Network (FSRN) we have worked extensively with food businesses to identify the 'problems worth solving by us', as described through their lived experience at the front line of food systems, and which exemplify an understanding and perspective on the prioritised issues and the solutions that are actionable in production settings and food policy. Case studies will be presented on how collaborative projects have been established, funded, and are now delivering key outcomes related to technology proofs-of-concept, development of new food safety guidance and training, and establishing the needs for new models for microbiology data sharing.

From Chance Meeting to Startup – The Journey of Commercialising Novel Biobased Textile Fibres

James MacDonald

Solena Materials Limited, London, United Kingdom

Abstract

The transition from academic research to commercialisation is often unpredictable, shaped as much by serendipity as by strategic planning. My journey began with a chance meeting at a networking event. At the time, I was a Research Fellow at Imperial College London, working on computational protein design with no focus on materials. However, an unexpected conversation at a DSTL event on synthetic biology for materials applications set in motion a chain of events that led to a successful proof-of-concept project, a contract with a global fashion brand, and ultimately the spin-out of Solena Materials. This talk will reflect on the path from fundamental research to commercial application, highlighting the role of interdisciplinary collaboration, funding opportunities, and the unforeseen moments that can shape scientific careers.

Careers Session: Transitions: Career Paths Outside of Academia

Navigating the transition to different career destinations with your SKEI bag

Yinka Somorin ORCID iD

RBridge Research and Advisory, Accra, Ghana. Ajayi Crowther University, Oyo, Nigeria

Abstract

The world of work is changing globally and driven by technological advancements, shifting economic paradigms, and evolving societal values. Traditional career pathways are becoming uncommon due in part to the changing world of work, as well as life circumstances and personal choices. Researchers increasingly face the challenge of adapting to emerging opportunities and how to navigate the transition to new career destinations. To overcome this challenge, some universities are focusing on equipping researchers with diverse knowledge and skills to make career choices suitable for them. Despite the growing resources available for making career decisions, many researchers are still unaware of the options available to them and how to maximise these opportunities. Reflecting on my career journey as a microbiologist, I will draw insights into how microbiologists can plan and prepare for career transitions, from where they are. The talk will highlight the different roles microbiologists have gone on to, the importance of creating an inventory of their skills and experiences, and how to adapt these to different career opportunities. Finally, this talk will address the emotional transitions and mind shifts required for making these career transitions.

From Academia to Industry: My Journey in Science and Innovation

Aurore Poirier ORCID iD

Vidiia Ltd, Guildford, United Kingdom. University of Surrey, Guildford, United Kingdom

Abstract

In this talk, I'll share my career journey starting from my academic beginnings in microbiology and parasitology to my current role as Head of Science at VIDIIA, a MedTech start-up company. Along the way, I've had the opportunity to work on fascinating projects, from developing a biomedical device to treat sepsis during my first postdoctoral experience, to participating in international collaborations on rapid molecular diagnostic solutions for infectious diseases, and, most recently, in the development and commercialisation of an AI-assisted diagnostic platform.

The transition from academia to industry was a pivotal moment in my career. While it brought new challenges, it also offered opportunities to apply my scientific expertise in practical, impactful ways. Through these experiences, I've learned to navigate multidisciplinary environments, adapt to fast-paced innovation, and leverage the transferable skills I gained in research. I've also discovered the value of building meaningful collaborations across academia, industry, and international teams.

In this session, I'll reflect on key lessons learned during this journey, including strategies for embracing change, overcoming challenges, and thriving in diverse roles. I'll also share practical advice for attendees who may be early in their careers, considering a transition from academia, or curious about how to make an impact in both research and industry. My aim is to provide an honest perspective on creating a fulfilling career at the intersection of science, technology, and innovation.

Pivoting from Pipettes: A Scientist's Move to Sales

Robyn Elizabeth Braes ORCID iD

Novogene (UK) Company Limited, Cambridge, United Kingdom

Abstract

I always saw myself having a career in academia. I loved the lab, research, and contributing to the science through my work in microbial genomics. As time went on, I discovered that while I had a passion for science, I was equally drawn to connecting with people. After finishing my PhD, I looked beyond academia and landed in a role I never expected, as an Account Manager.

Using my academic background, I now work as a sales representative for Novogene Europe, a genomic sequencing company based in the UK. During this session I will share how I went from inoculating flasks and troubleshooting PCRs, to helping researchers find the sequencing services they need to push their own work forward.

I will share my experiences on stepping out of the lab, the challenges of learning the ropes in sales, and how my science background gave me an edge in my new career.

I will also cover misconceptions about moving into industry—spoiler alert: you don't need to leave the science behind. Instead, I have found new ways to use my knowledge, collaborate with researchers, and stay connected to the microbiology world, just from a different angle.

If you are thinking about a switch to industry, or curious about life outside of academia, I hope this talk gives you inspiration as I share the ups, downs, and lessons I have learnt along the way. Turns out, there is a lot of life—and science—beyond the lab bench!

From Benchside to Business: My Career Journey in Life Sciences & Genomics

Shane Houston

BMKGENE (BioMarker) Europe, Münster, Germany

Abstract

A concise look at how my career journey evolved from a lab-based research role to consultative scientific sales, blending scientific expertise with strategic business insights in the life sciences and genomics industry.

There and almost back again- a microbiologist's tale

Helen McNeil ORCID iD

MicrobesNG, Birmingham, United Kingdom

Abstract

We've all heard the phrase 'failing to plan is planning to fail' but somehow I've never been able to apply that to my career. This is the story of how I followed my feet and found myself with skills that I was able to use in my current role at MicrobesNG. I hope it will inspire you to do what you enjoy and forget about imposter syndrome!

From the lab to law: a career in science and intellectual property

Richard Gibbs

Marks & Clerk LLP, Glasgow, United Kingdom

Abstract

This talk will summarise the journey from science degree to Chartered UK and European Patent Attorney. It will discuss how a microbiology background provides a solid platform for a career as a Patent Attorney and will offer advice for anyone thinking about joining the IP profession. We will also look at what the job entails, the types of client we get to interact with, the exams (!) and the career options available within the private and industry sectors.

Champions of Change: Celebrating Actions Advancing Equality, Diversity, and Inclusion

Community College Active Research Equity Initiative (CCAREI): Increasing Access to Research Experiences in Historically Underrepresented Student Communities

Skylar Gay ORCID iD, Charlie Gleek

University of Virginia, Charlottesville, USA

Abstract

The Community College Active Research Equity Initiative (CCAREI) is a program aimed at increasing research opportunities for community college students by fostering collaborations between faculty at community colleges and four-year institutions. Community college students are often excluded from meaningful research experiences, limiting their career prospects in STEM fields. CCAREI addresses this gap by facilitating partnerships that allow community college faculty to engage in active research and course design alongside university faculty.

CCAREI's pilot program, hosted by the University of Virginia (UVA), enables community college faculty to conduct research in UVA laboratories over the summer, focusing on cutting-edge topics such as microbiology, antibiotic resistance, and biofilm formation. Participants also attend UVA's Course Design Institute to incorporate research-based learning into their curricula, ultimately enriching the educational experiences of their students.

The initiative aims to create long-term, sustainable research collaborations between institutions, thereby broadening research participation among students from diverse socioeconomic and academic backgrounds. By focusing on equitable research opportunities and the professional development of faculty, CCAREI aspires to reshape the landscape of STEM education, providing community college students with the tools and mentorship they need to thrive in research-driven careers.

Mums in Science: A 17-Year Journey Supporting Women in STEM

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

Since its founding in 2005, the Mums in Science network (https://mumsinscience.org) has grown to support over 15,000 women across 40 countries, making it a vital resource for women in STEM. Despite women making up around 50% of university graduates in STEM, only 30% continue their careers in research after family responsibilities arise, with even fewer women advancing to senior roles. Mums in Science addresses these challenges by offering two free monthly events focused on building soft skills, such as leadership, resilience, and effective communication—essential for navigating the workplace. Our workshops have been approved by the Royal Society of Biology for purposes of CPD points. By fostering personal and professional development, this network helps women stay connected and thrive in their STEM careers. This presentation invites participants to learn from our history and explore how similar initiatives can be launched to promote equity, diversity and inclusion in microbiology and beyond.

Education and Outreach Symposium

Invited: Education, outreach and research – the magic is in the mixture!

Jody Winter

Nottingham Trent University, Nottingham, United Kingdom

Abstract

While supporting students with research projects studying the prevalence of antimicrobial resistant bacteria in local rivers, it quickly became apparent that we were missing a lot of information and opportunities. Without broader contextual information, we could not fully interpret our data. Did the bacteria we were detecting pose any risk to human health? What should we tell people about our findings?

We redesigned our project to centre outreach, community engagement and citizen science, to help make sure we would be asking the right questions and gathering informative data. In this talk, I will discuss our "to paddle or not to paddle – what's in our river?" project. This involves surveying community members on where and how they access local waterways, involving them in water sampling, and longitudinal analyses of antibiotic susceptible and resistant faecal indicator bacteria (FIB) and associated physicochemical parameters at "hot spot" sites where FIB and potential human exposure levels are both high. Ultimately, we aim to generate useful research data supporting the development of quantitative risk assessments for recreational users of waterways, while also engaging members of our local community in scientific research and raising public awareness of microbiology and antibiotic resistance.

Invited: ❤ Flipping inclusive bioscience education: unlocking co-created classrooms for all ❤ *(Please participate in the fun pre-activity and competition: details in the abstract)*

Jo Rushworth ORCID iD

University of Lincoln, Lincoln, United Kingdom

Abstract

The awarding gaps that persist in Higher Education are a shameful reminder that we are still far from creating an equitable bioscience classroom. Having been a disabled, mature, international and widening participation student myself, I have experienced personally what I call the *"student experience"* gap, or *"this isn't my classroom"*, which I believe underpins inequalities in student outcomes.

I'd like us to have an engaging and interactive session where we explore and share ideas around flipping bioscience education using inclusive co-creation. Let's explore how Universal Design for Learning (UDL) can help to inspire and frame more inclusive and flexible microbiology curricula where every learner can see themselves represented and can engage and flourish. Let's delve into the benefits of what some may consider the radical idea of students and staff swapping roles. Finally, let's look at a very different pedagogic approach which takes its roots from language acquisition and music education, where there are no attainment gaps. Could we apply this to microbiology education?

Before the session, please have a go at the fun pre-activity below. Can you crack the code?
https://padlet.com/jrushworth1/flipping-inclusive-bioscience-education-have-we-cracked-it-86c6he3a60nqmr3p

There's a prize to be won, an opportunity for you to tell me about yourself and your own learner experiences which will shape the session, and you can use the Padlet to meet others and work together on the activity before the conference.

So, please come ready to participate, have some fun and hopefully come away with new ideas to try in partnership with your learners.

Decolonising University Curricula with Generative AI: Reducing Barriers and Increasing Engagement in Microbiology

Catherine Lawler[1,2], Tanny Saha[2]

[1]University of Birmingham, Dubai, UAE. [2]Bath Spa University, Bath, United Kingdom

Abstract

The decolonisation of curricula in Higher Education seeks to address enduring colonial legacies in Western university education. This study assessed colonisation within a Microbiology curriculum, explored the impact of decolonisation on undergraduates, and identified barriers faced by teaching staff. Both colonised and decolonised resources were developed and tested through two mixed-method surveys. Results showed that undergraduates felt greater engagement and belonging with decolonised materials, while staff cited time and training as major barriers to decolonisation efforts. To address the time constraint, Generative AI (GenAI), specifically ChatGPT-4, was utilised to assist staff in developing decolonised resources, reducing preparation time by 90%. Students displayed no preference between resources created solely by staff and those aided by GenAI, although staff emphasized the need to maintain scientific rigor. This study highlights the potential of GenAI to facilitate decolonisation, and therefore enhance student belonging in Microbiology teaching.

‘Feeling the code’: emotional responses in diverse introductory programming courses and simple interventions to create more emotionally sensitive learning environments

Liam Cremona ORCID iD[1,2], Niall Dunne[2], Archana Sharma-Oates[2], Phillip Smith[2], Lindsey Compton[2]

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

Abstract

Aims. Universities and disciplines may differ, but all face a similar set of challenges in teaching computer programming languages, notably an emotionally charged response to learning to code. The overall aims of this project were to understand the emotions students experience in an online distance learning introductory programming module and evaluate how student emotions influence different learning strategies, affect student motivation and engagement and ultimately course outcomes (performance).

Methods. We engaged with students on MSc Bioinformatics programmes delivered either on campus, or Distance Learning (DL) and compared with the MSc Data Science, using surveys and focus groups to explore the emotions students experience when learning to code in various languages, including bash, Python and R.

Key findings. Students can find learning to code frustrating, particularly as a result of a mismatch between expectation and reality, so we need to focus on assessing the *process* as well as the *outcome* of their learning experience. Prior coding experience was significantly associated with reduced negative emotional responses to coding, while females tended to express more negative responses to coding than males. The emotional negativity score did not differ significantly by programme and showed no clear relationship with student performance. Based on our findings, we developed simple interventions based on humanizing the learning experience to make the learning experience more positive.

Practical recommendations. Student expectations need to be managed upfront, involving normalising the experience of learning to code, including the need to make copious mistakes, take an iterative approach and adopt a growth mindset.

Maintaining good oral health reduces the impact of antimicrobial resistance: An outreach project in rural communities in Kenya

Kitty Guo ORCID iD, Beth Heaney, Priya Purewal, Orla O'Brien

University of Dundee, Dundee, United Kingdom

Abstract

Background: Dental students, as future antibiotic prescribers must engage in antimicrobial resistance (AMR) stewardship. This project, co-lead by 4th year dental students aimed to deliver important AMR messages to remote communities in Kenya as well as encourage dental students to consider the global effects of AMR.

Methods: In collaboration with a local charity, Ace Africa, and global AMR network ReAct, students from Dundee Dental School delivered oral health education and advice to remote and rural communities in Kenya. This included visits to schools, health centres, vocational training centres and village meetings.

Results: The team engaged 5926 school children and 75 school teachers from 18 schools in Siaya and Busia counties. 213 community members were engaged in village meetings. 4 health centres/ dispensaries were visited, delivering training to 53 healthcare workers and 528 members of the community. 1500 students and 20 staff members were engaged in training at a Young Mothers' vocational training centre. From the schools, pre and post session feedback identified an improvement in the understanding that antibiotics do not cure toothache. This project raised the profile of the limited access to dental care in these remote communities to the Department of Health, Siaya County.

Conclusion: This project delivered important oral health information to communities in remote areas of Kenya, where access to dental treatment is limited and AMR related health issues are a significant concern. It also engaged future antibiotic prescribers to consider the global consequences of AMR.

Transforming Master's Research in Microbiology: A Collaborative Approach to Enhance Student Learning and Resource Efficiency

Monika Gostic ORCID iD, Erskine Lynda, Bin Hu, Samantha Miller, Ian Stansfield

Aberdeen University, Aberdeen, United Kingdom

Abstract

This abstract presents an innovative pilot study conducted at the University of Aberdeen during the 2023-2024 academic year, which reimagined the structure of Master's research projects in microbiology. The pilot implemented a collaborative model that grouped 13 students into four thematic project areas, each addressing different aspects of microbiology and biotechnology. This approach pooled individual funding to support a cohesive, group-based research experience and employed a rotating supervision model involving four supervisors. The project utilised digital tools like Microsoft Teams to facilitate communication and collaboration. Student feedback was overwhelmingly positive, with an average satisfaction rating of 9.14 out of 10 and a unanimous recommendation of the program to future students. This collaborative approach offers several advantages, including enhanced peer learning, improved collaboration skills, exposure to diverse research methodologies, and a more supportive learning environment. Additionally, the model demonstrated significant operational benefits, including more efficient use of laboratory resources, reduced overall resource consumption, and freed staff time through shared supervision responsibilities. The pooling of individual funding allowed for more ambitious research projects while optimizing resource allocation. This approach not only enhanced the student learning experience but also improved the overall efficiency of the research program. The success of this pilot study suggests that this model could be effectively scaled and adapted to other institutions, potentially transforming how we prepare the next generation of microbiologists for the realities of modern scientific research while simultaneously addressing resource constraints in academic settings.

Making biomedical final year projects more relevant to life science employers

Georgios Efthimiou

Centre for Biomedicine, Hull York Medical School, Hull, United Kingdom

Abstract

Final year projects in higher education are a very important interface between academic and professional life, as students gain many crucial skills and develop several key qualities that are sought after by many employers in the area of life sciences, and beyond. Since 2022, we have made major modifications to our final year project module, aiming to make this student experience more relevant to employers, who have certain job market demands and expectations for their prospective graduate-level talent pools. These included: (a) Providing a clear and standardised structure for project timelines; b) Consolidating basic lab techniques, such as pipetting, light microscopy, aseptic technique, buffer preparation and molarity calculations; (c) Introducing group training in advanced lab techniques, such as qPCR, flow cytometry, fluorescent microscopy and western blotting, provided by our technical staff team; (d) Creating an endorsed and discoverable skillset via use of LinkedIn; (e) Implementing a virtual company environment, with regular lab meetings with industrial partners, virtual rewards, professional quality requirements and virtual client-driven experimental objectives. Feedback from students on these interventions was very positive, with more than 75% of our biomedicine final year students finding them creative, enjoyable and 0helpful for their academic performance and future employability. Staff satisfaction was also high (68%). With these module improvements, we aspire that our graduates will be better prepared for the challenges of the job market, armed with skills and competencies that will attract the attention of future employers and increase their employability and the probability of a successful career.

Co-Creation of Accessible Microbiology Practicals to enhance Skills Gaps in Biosciences Graduates

Sean Goodman ORCID iD, Sophie Haslam, Amy Wedley ORCID iD, Gemma Wattret ORCID iD, Rebecca Verspoor, Jo Fothergill ORCID iD, Nicola Williams ORCID iD, Rachel Floyd

University of Liverpool, Liverpool, United Kingdom

Abstract

Addressing the critical “One Health” challenges, such as antimicrobial resistance, food security, and biodiversity loss, requires a workforce with robust practical skills in microbiology. However, gaps between theoretical knowledge and practical application persist in Life Sciences education, as employers report graduates lack certain career-relevant competencies. In response, this student-led project aims to co-create an accessible microbiology curriculum that bridges this gap, integrating sustainable laboratory and fieldwork practices with real-world application. Through collaboration between students and staff, this project has developed a module that focuses on the development of a pipeline of sample collection, cultivation, use and genomic exploration and includes protocols to minimise single-use plastics, optimize sample stability, and enhance inclusivity in field sampling and laboratory research. Digital resources, including immersive virtual site tours created with Insta360 action cameras, allow students who face physical, wellbeing or ethical barriers to engage in fieldwork experiences remotely.

The overall impact of this project was measured through a qualitative and quantitative mixed methods evaluation that has been designed to encapsulate views on student learning, engagement, employer value and overall satisfaction, and supports Education for Sustainable Development (ESD) goals and is in line with the University’s commitment to Net Zero by 2035. By embedding digital fluency, fieldwork, cross-disciplinary skill sets and critical “One Health” competencies into the microbiology curriculum, this project prepares students to tackle pressing public health, biotechnological, food security and ecological challenges. Our approach offers a scalable template for other programs aiming to foster inclusive, practical, and sustainable education across the health sciences.

Enteric Bacteria; Biology, Diversity & Ecology

Invited: The wonderful world of *Escherichia coli*

Nicola Holden

SRUC, Aberdeen, United Kingdom

Abstract

Escherichia coli is perhaps the best studied species in all of biology. Whether as a model for our own physiology and metabolism, a major workhorse of molecular biology and biotechnology, a genetic reference and biological baseline for experimentation, or as a pathogen, there's not a lot we don't know about *E. coli*. But the trouble is that we only ever see a small proportion of what the species, or indeed the species complex, is capable of. And yet, this species uses all of the potential that being a mesophile with wide metabolic flexibility can offer. What's needed is a step back to appreciate where it is, what it does and how. This talk aims to provide a broader understanding of our humble friend, taking examples from the wider environment, to give it a bit more appreciation the next time you streak out a plate or grab a bit of sequence.

Genomic epidemiology and phenotypic characterisation of *Salmonella enterica* serovar Panama in Victoria, Australia

Samriddhi Thakur ORCID iD[1,2], Sarah L. Baines[1], Cheryll M. Sia[3], Mary Valcanis[3], Louise M. Judd[4], Benjamin P. Howden[1,3,4], Hayley J. Newton[2], Danielle J. Ingle[1]

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Abstract

Salmonella enterica serovar Panama, a causative agent of non-typhoidal salmonellosis (NTS), has been significantly associated with invasive NTS disease (iNTS) in humans. *S.* Panama is an understudied pathogen, with its pathobiology poorly understood. It is a predominant iNTS serovar in Australia, a high-income country with high rates of salmonellosis. This study investigates the genomic epidemiology and infection biology of *S*. Panama isolates recovered in Victoria, Australia, between 2000 and 2021. Two sub-lineages, encompassed within a previously described Asian lineage, were identified. Multi-drug resistance (resistance to ≥3 drug classes) was detected in 46/89 (51.7%) Australian isolates. The plasmid-mediated colistin resistance gene, *mcr1.1*, was detected in one Australian *S.* Panama isolate, carried by an IncI plasmid previously reported in *Salmonella* and *Escherichia coli* isolates from poultry in South-East Asia. Examination of the intracellular replication dynamics of seven *S.* Panama isolates, representing the genetic diversity of the study population, demonstrated diverse phenotypes. In THP-1 derived macrophages, despite low host cell uptake, *S.* Panama showed higher replication rates over time compared to *S. enterica* serovar Typhimurium SL1344 strain. However, a causative genomic mechanism could not be identified to explain this observed phenotype. This study provides insights into the *S.* Panama isolates imported into Australia over two-decades showing MDR was common in this iNTS serovar and colistin resistance reported for the first time. It provides the first data on the host-pathogen interactions of *S.* Panama in Australia, which will aid our collective understanding of the pathobiology of *S*. Panama and iNTS serovars more broadly.

Exploration of Bacterial Evolution and Dynamics in a Natural Two-host Setting

Hope Okon ORCID iD[1], Hayley Thompson ORCID iD[1], Gonçalo Silva ORCID iD[1], Steven Belmain ORCID iD[1], Richard P. Smith ORCID iD[2], Hannah May[2], Judy Bettridge ORCID iD[1]

[1]University of Greenwich, Chatham, United Kingdom. [2]Animal and Plant Health Agency, Surrey, United Kingdom

Abstract

Salmonella Typhimurium has traditionally been considered the archetypal broad host-range serovar, although growing evidence indicates it should be considered as a collection of pathovariants that differ significantly in their degree of host-adaptation. During transmission, the bacteria are continually faced with biotic and abiotic conflicts that influence their adaptation as they interact with both wildlife and livestock hosts, suggesting the need to understand their evolutionary dynamics in natural systems.

Pig farms are frequently infested with large, dense rodent populations, due to the abundant food, water and shelter. Rodents are known to contract, maintain and spread *Salmonella* between batches of pigs leading to persistent infection even after disinfection of farms.

The study aims to investigate *Salmonella* diversity and evolutionary dynamics in a natural two-host system by comparing the genomes of *Salmonella* cultured from rodent and pig faeces on a single farm, collected over a one-year period.

All cultured isolates of *Salmonella* were sequenced using the Illumina MiSeq platform, and serovars were predicted. Overall, 26.9% (197/732) of pig samples and 13.6% (15/110) of rodent samples were culture-positive for *Salmonella*. Ninety-six percent (203/212) of the total isolates were monophasic *Salmonella*Typhimurium (mST) from both hosts. Other serovars identified were *Salmonella* Cerro (1/212) and *Salmonella* Newport (1/212) from rodents, and rough *Salmonella* (7/212) from the pigs. The mSTs are being investigated for within-serovar diversity to observe how their pangenomes, antimicrobial resistance and virulence genes differ by host species and time. Findings could inform genomic surveillance efforts for this important foodborne pathogen.

Needle Amnesty: Aurodox inhibits the Type III Secretion System of multiple Gram-negative organisms

David R. Mark ORCID iD[1], Nicky O'Boyle ORCID iD[2], Kabo Wale ORCID iD[1,3], Samantha Tucker University of Glasgow ORCID iD[1], Rebecca McHugh ORCID iD[1], Andrew Roe ORCID iD[1]

[1]University of Glasgow, Glasgow, United Kingdom. [2]Trinity College Dublin, Dublin, Ireland. [3]University of Botswana, Gaborone, Botswana

Abstract

The Type III Secretion System (T3SS) is a needle-like multiprotein complex, employed as a virulence factor by Gram-negative human, animal, and plant pathogens. This makes the system an attractive target for developing antivirulence compounds to attenuate infections *in lieu* of antibiotic treatment. Aurodox, a polyketide natural product of *Streptomyces goldeniensis,* has previously been shown to downregulate expression of the locus of enterocyte effacements (LEE), the T3SS of *E. coli* O157:H7 in a *ler* dependent manner, and to protect epithelial cells from colonisation by the bacterium. However, questions remained as to whether or not aurodox's antivirulence effect translated to other organisms.

Here, we show that aurodox does affect multiple Gram-negative T3SSes. Using RT-qPCR, we tested the effect of aurodox on the expression of 5 T3SSes encoded by *Yersinia pseudotuberculosis* (1 T3SS)*, Vibrio parahemolyticus* (2 T3SSes)*,* and *Salmonella* Typhimurium (2 T3SSes), and showed that aurodox inhibits some, but not all T3SSes. A phylogenetic tree we constructed using core T3SS component genes suggests that the effect of aurodox is restricted to SPI-2 type T3SSes. In addition, we use GFP transcriptional reporter assays and RNA-Seq to show that aurodox prevents the induction of SPI-2 in *Salmonella* Typhimurium *in vitro*. Aurodox also reduced the ability of *Salmonella* Typhimurium to survive intracellularly within RAW 264.7 macrophage like cells.

This work provides nuance to our understanding of the mechanism of action of aurodox – although it did not inhibit all T3SSes tested, it nevertheless was able to attenuate virulence in *Salmonella* Typhimurium in a cellular model of infection.

Modulation of a carcinogenic bacterial genotoxin in E. coli bowel isolates

Emily Addington ORCID iD[1], Sofia Sandalli ORCID iD[1], Islay Kamraoui[2], Andrew Roe[1]

[1]University of Glasgow, Glasgow, United Kingdom. [2]Université de Montpellier, Montpellier, France

Abstract

Strains of *E. coli* residing in the human intestinal tract may carry the *pks* genomic island, an operon which encodes the biosynthetic machinery for the production of the cyclomodulin and genotoxin, colibactin. Colibactin damages DNA and promotes chromosomal instability to directly induce tumorigenesis in colorectal cancer (CRC), the second-leading cause of global cancer-related deaths. Patients with intestinal bowel diseases such as Crohn's and ulcerative colitis have an increased risk of CRC, and it has been proposed that suffers carry a higher proportion of B2 phylogroup and *pks+ E. coli* than healthy individuals. Our research shows that the D-enantiomer of the amino acid serine is capable of modulating colibactin production at a transcriptional level, acting on the *clb* genes required for genotoxin biosynthesis. D-Serine supplementation of colibactin-producing *E. coli* strains, including widely used probiotic Nissle 1917 and *pks+* Adherent-Invasive *E. coli* clinical isolates from diseased bowls, can either enhance or repress colibactin production in a strain and media-dependent manner. Transcriptomics were performed to decipher interactions of D-Serine with the *clb* locus, and our results provide insight into how and why the *clb* operon response to D-Serine supplementation differs across *pks+* strains under varied conditions. Our research elucidates the complex regulation of the colibactin locus and the mechanism of action by which D-Serine can modulate genotoxin production. Such understanding of how possible therapeutic or dietary components can impact the production of the genotoxin are critical for prevention and treatment of colibactin-induced CRCs.

Genomic Insights of Scottish *E. coli* Source Attribution and Antimicrobial Resistance

Antonia Chalka ORCID iD[1], Louise Crozier[2], Adriana Vallejo-Trujillo[1], Vesa Qarkaxhija[1], . PATH-SAFE Partners[3], Jacqui Mcelhiney[2], David Gally[1]

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Abstract

Our study presents novel source attribution models developed using whole genome sequences of *E. coli* from a substantial collection of isolates, primarily sourced from Scotland. The isolates encompass a diverse range of origins, including wastewater, human clinical, livestock, canine clinical, food, and shellfish samples, resulting in a comprehensive 'one health' dataset of 4,230 high-quality genome assemblies with accompanying antimicrobial resistance (AMR) genotypic data. This research evaluates the utility of source attribution models in a one health public health surveillance framework. Models based on Random Forest algorithms achieved more precise attribution than those relying on phylogenetic analysis alone, enabling probabilistic identification of contamination sources in food and water samples. Additionally, the study investigates how wastewater isolates can enhance predictions of human association, with food isolates demonstrating significant human attribution and livestock attribution aligning with anticipated host sources. AMR findings reveal a gradient of diversity, with high AMR complexity in human clinical isolates and lower levels in ruminant isolates, including reduced AMR allele frequencies in human-associated isolates from environmental sources. This research highlights both the complexities and promise of leveraging a highly diverse sentinel species to monitor bacterial and AMR transmission within a one health context.

Culturing of previously 'unculturable' rumen bacteria using culturomics with dilution to extinction and direct plating methods on diverse media

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Abstract

The rumen microbiome allows ruminants to metabolically access the energy stored in plant material. This diverse ecosystem includes anaerobic bacteria, protozoa, fungi, archaea, and bacteriophages. The complexity of culturing anaerobic microbes, coupled with the high cost, time, and skilled labour traditionally required for their identification, has resulted in limited culture collections from the rumen. In this study, we employed a culturomics approach to isolate previously unculturable bacteria from rumen fluid samples collected from dairy cattle at different ages (12, 28, and 49 weeks) and managed under conventional or intensive systems. We used two isolation methods (direct plating and dilution to extinction) and two media types (Hobson's M2 and BHI with rumen fluid supplementation), all under anaerobic conditions at 39°C to mimic the rumen environment. The culturomics approach resulted in the isolation of 331 pure rumen bacteria identified with 16S rRNA gene sequencing undertaken by Sanger sequencing. A BLAST search against the Greengenes2 database showed that the isolates belong to 7 phyla—*Actinomycetota*, *Bacillota*, *Bacteroidota*, *Desulfobacterota*, *Fusobacteriota*, *Pseudomonadota*, and *Spirochaetota*—encompassing a total of 59 species. Additionally, 62 isolates had less than 97% similarity, indicating they may represent potentially novel rumen bacteria and contribute to closing the gaps in rumen unculturable bacteria research. Finally, 23 co-cultures were obtained; their identification will provide insights into microbial interactions within the rumen. This study enhances our understanding of the rumen microbiome and provides potential for biotechnological applications, such as developing direct-fed microbials (DFMs) to redirect hydrogen during fermentation away from methane pathways.

Environmental, Applied & Industrial Microbiology Forum

Understanding *Bacillus* spore interactions on textiles for innovative cleaning technologies

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Abstract

Textile production and maintenance has a wide range of environmental impacts including energy, water and chemical consumption and the generation of landfill waste. Changes to laundry habits are occurring slowly, with average wash temperatures for the UK and Germany decreasing from ~60°C to 40-46°C. However, further reductions in wash temperature conditions and frequency are required to improve garment life cycles by maintaining higher textile quality for longer. This will reduce carbon emissions associated with the laundering process.

Bacillus spores are being investigated as a biotechnology to improve item cleaning; previous studies have proven the initiation of soil breakdown since upon germination the vegetative cells excrete extracellular enzymes. Although there is a wealth of knowledge focused on adhesion of *Bacillus* spores to surfaces in the food industry, information regarding deposition on textiles warrants further investigation.

This project aims to mechanistically understand *Bacillus* spore interactions and the physicochemical properties of specific textiles. Bioimaging techniques will be utilised, with the possible development of bespoke software, to assess textile and spore interactions. Upon deposition, the focus will shift to the identification of enzyme production and the efficacy on soil removal for textiles washed in quick and cold conditions.

Investigating antimicrobial stress response in Escherichia coli induced by high-frequency ultrasound

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Abstract

Mild non-thermal processing techniques such as ultrasound are increasingly explored as alternatives to conventional methods for microbial inactivation in food safety applications. However, sublethal stresses induced by these treatments may activate bacterial stress responses, potentially leading to the development of antimicrobial resistance (AMR). This study investigates the effects of high-frequency ultrasound (500 and 760 kHz) on *Escherichia coli*, with particular focus on oxidative and heat stresses, which were applied both individually and in combination.

Ultrasound treatments were applied for 10, 20 and 30 min at two frequencies. The generation of ultrasonic-induced hydrogen peroxide (H_2O_2) was measured, and the temperature increase during treatment recorded. These parameters (temperature and oxidative stress) were subsequently applied to assess their individual and combined effects on bacterial stress responses. Results were compared to bacterial inactivation achieved by ultrasound alone to understand whether sublethal conditions induced by ultrasound contribute to AMR development or if a synergistic effect is present that enhances microbial inactivation.

The findings indicate sublethal heat stress plays a significant role in promoting bacterial resistance. This suggests that inadequate control of ultrasound treatment conditions could inadvertently contribute to the survival and adaptation of pathogens such as *E. coli*. The combination of oxidative and heat stress, however, may offer a more effective strategy for microbial inactivation. These insights emphasize the necessity for the optimisation of ultrasound parameters to prevent the unintended promotion of resistance while ensuring effective microbial control in food processing systems.

Developing a commercial bacteriocin-based treatment for protecting potatoes against Blackleg disease and soft rot

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Abstract

Potatoes are a critical global crop and are highly susceptible to bacterial diseases. In the UK *Pectobacterium atrosepticum* is the main cause of blackleg disease and soft rot, resulting in annual losses of approximately £50 million. Infection is exacerbated by weather, particularly increased rainfall, and there are currently no effective treatments, relying solely on field inspections for control.

The economic impact of Blackleg is severe, with climate change and flooding further damaging crops and reducing harvests. As extreme weather becomes more common, it is essential to explore alternative methods to manage *Pectobacterium* infections.

We are developing a bacteriocin-based strategy to control *P. atrosepticum* infections. Bacteriocins are narrow-spectrum antimicrobial proteins produced by bacteria that target closely related species -. Our focus is on creating a prophylactic treatment for blackleg disease using selected bacteriocins.

We have identified and synthesized several promising candidates effective in killing nearly all UK strains of *P. atrosepticum.* We are testing their efficacy against *P. atrosepticum*, using an image-based potato tissue infection assay and by glasshouse trials with seed tubers. Our first candidate has shown very encouraging results in tissue infection assays, reducing infection by 85-90% at inoculation titres equivalent to field-infected tubers, and in glasshouse trials using whole tubers. This demonstrates its potential for use as a treatment to suppress *P. atrosepticum* infection.

This innovative approach addresses the urgent need for effective control measures. We aim to develop it into a commercially viable treatment, ensuring the sustainability of potato production amid increasing environmental challenges.

Engineering myxobacterial super-predators to fight crop disease

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Abstract

Myxobacteria hold untapped potential as biocontrol agents to protect vital cereal crops from the devastating effects of fungal diseases. Often considered as a keystone taxon in the soil microbial community, myxobacteria have extremely wide prey ranges (including bacterial, oomycete and fungal prey) and as apex predators can profoundly influence the soil microbial community. *Zymoseptoria tritici* causes septoria leaf blotch - a fungal disease of wheat that can substantially reduce crop yield, having severe economic impacts. We have used *in vitro* assays to demonstrate the predatory abilities of a range of myxobacterial strains against *Z. tritici*¸ including the ability of some to impact biofilm formation, evidencing the potential for myxobacterial strains to be utilised as biocontrol agents against *Z. tritici*. In addition, we have taken a bioinformatics approach using myxobacterial pangenomics and a genome-wide association study (GWAS), to reveal individual myxobacterial genes with a significant correlation with predatory activity. These genes of interest are ideal candidates for gene editing with the aim of producing one or multiple engineered strains of myxobacteria with enhanced predatory activity. Such 'super-predators' will likely show improved predation against *Z. tritici* and contribute to the formulation of a biocontrol treatment for use in wheat plants, which may confer prophylactic and/or therapeutic effects against the widespread effects of septoria leaf blotch.

Polystyrene nanoparticles induce biofilm formation in *Pseudomonas aeruginosa*

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Abstract

In recent years, micro/nanoplastics have garnered widespread attention due to their ecological risks. In this study, we investigated the effects of polystyrene nanoparticles (PS-NPs) of different sizes on the growth and biofilm formation of *Pseudomonas aeruginosa* PAO1. The results demonstrated that exposure to certain concen- trations of PS-NPs significantly promoted bacterial biofilm formation. Meanwhile, we comprehensively revealed its mechanism whereby PS-NPs induced oxidative stress and altered bacterial membrane permeability by con- tacting or penetrating bacterial membranes. To counteract the stimulation by PS-NPs and reduce their toxicity, bacteria enhanced biofilm formation by upregulating the expression of biofilm-related genes, increasing EPS and virulence factors secretion, and enhancing bacterial motility through the participation of the quorum sensing (QS) system. Additionally, we also found that exposure to PS-NPs enhanced bacterial antibiotic resistance, posing a challenge to antimicrobial therapy. Our study reveals the toxic effects of nanoplastics and the defense mech- anisms of bacteria, which has important implications for the risk assessment and management of environmental nanoplastics.

Harnessing Horizontal Gene Transfer for Bioremediation: Functionalized pQBR57 plasmid drives terephthalic acid degradation, conjugation, and plasmid stability in soil microbial communities

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Abstract

Horizontal gene transfer (HGT) enables bacterial communities to adapt rapidly to fluctuations in the environment. Genetic bioaugmentation is a bioremediation technique that uses the processes of HGT to introduce genetically-encoded bioremediation traits to microbial communities *in situ*. The environmental conjugative plasmid pQBR57 is a great candidate for genetic bioaugmentation due to its high stability, conjugation rate and broad host range. The plasmid was functionalized with the bioremediation trait of terephthalic acid degradation via the integration of an engineered tphKAB operon. A model soil microbial community, composed of 128 soil-isolated bacteria, was metabolically characterized and taxonomically typed (16S rRNA). The community was divided into four community compositions: (i) no community, (ii) 64 pseudomonads, (iii) 64 non-pseudomonads, and (iv) complete community. Each community included *P. fluorescens* as the plasmid-bearing donor and *P. putida* as a potential recipient. The plasmid-bearing donor carried either the wild-type pQBR57 or the functionalized pQBR57-tphKAB. Serial transfer every 4 days of 1% of the community into polluted (3.2 mg terephthalic acid per gram of soil) or non-polluted soil microcosms were performed for 8 transfer (32 days).

The functionalization of pQBR57 led to significantly greater plasmid density and HGT (measured by transconjugant density) in polluted soils across all community compositions. In addition, pollutant degradation by the functionalized plasmid was observed in all communities – 53%, 36%, 27% and 21% pollutant degraded in the final transfer, respectively. These findings emphasize the potential of environmental plasmids, such as pQBR57, to horizontally spread bioremediation traits into autochthonous communities *in situ*.

Novel environmentally conscious antimicrobial smart release coatings for the reduction of healthcare-associated infections and antibiotic usage dependence.

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Abstract

Background: Surfaces in healthcare environments act as bacterial reservoirs, facilitating a transmission pathway for pathogens between individuals. Healthcare-associated infections (HCAIs) cause preventable public health issues, leading to avoidable resource usage and escalating economic burden. These outcomes are often worsened by antibiotic resistance. Antimicrobial surface coatings successfully reduce surface biofouling, and subsequent HCAI prevalence. **Aim:** The aim of this project is to develop novel antimicrobial surface coatings with smart release functionality and environmentally conscious material selection, to mitigate against resistance risk and the climate crisis, whilst also being mindful of suitability for industrial scale-up. **Methods:** The designed system has three main components, the active antimicrobials, antimicrobial storage nanocontainers, and the coating matrix. Antimicrobials have been selected for compatibility with the nanocontainers using chemical property prediction, characterised using Fourier-transform infrared spectroscopy (FTIR), and UV-VIS spectrophotometry, and evaluated through broth microdilution and crystal violet assays to determine minimum inhibitory concentrations (MICs) and minimum biofilm inhibitory concentrations (MBICs) respectively. Coating matrix formulations, using biodegradable biopolymers with functional additive inclusions are characterised through multiple evaluative techniques, including FTIR, goniometry, disk diffusion, and ISO22196 associated methods. **Results:** Desirable characteristics have been achieved in each component evaluation, including effective antimicrobial concentrations to inform suitable nanocontainer loading concentrations, and additives required to increase water resistance. Effective MIC and MBIC concentrations ranged from 1.25mg/ml to 3.75mg/ml. Promising coating system formulations have been identified for further evaluations at larger scales. **Conclusions:** These surfaces have potential to reduce HCAIs, associated antibiotic use, costs, and increase antibiotic usage longevity.

Harnessing Native Metabolism in *Pseudomonas umsongensis* GO16 for the Production of Plastic Alternatives

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Abstract

2,5-Furandicarboxylic acid (FDCA) is a biobased analog to terephthalic acid (TPA), a monomer of the ubiquitous plastic, polyethylene terephthalate (PET). FDCA can substitute TPA in PET, producing polyethylene 2,5-furanoate (PEF). However, the use of FDCA for plastic applications is limited by high production costs. Biotechnology offers an alternative production route by using fewer toxic reagents, milder conditions, and cheap, waste feedstocks for upcycling potential.

Pseudomonas umsongensis GO16, which can natively metabolise the PET monomers ethylene glycol (EG) and TPA as growth and energy substrates, has been used to upcycle these molecules to valuable compounds previously. Building on this ability, GO16 is being explored as a microbial chassis for the synthesis of FDCA.

A precursor of FDCA is 5-hydroxymethylfurfural (HMF). *P. umsongensis* GO16 possesses an *hmf* operon, enabling it to utilise HMF as a sole growth substrate, with FDCA as an intermediate. FDCA decarboxylase (HmfF) was identified as the enzyme responsible for converting FDCA to further metabolism. The gene was deleted, allowing GO16 Δ*hmfF* to convert 50 mM of HMF to 50 mM FDCA in 48 hours when growing on EG. FDCA production with TPA growth is poor however, at only 12 mM.

To improve HMF conversion efficiency, target native proteins were overexpressed to create a more potent biocatalyst. This generated a strain capable of totally converting 50 mM of HMF to FDCA in 24 hours when grown on TPA, EG and mock PET hydrolysate. Scale up work will now follow to maximise the productivity of this strain.

Atmospheric nitrogen fixation and restricted mobility of arsenic on plant root-associated diazotrophic biofilm enhance plant anatomy

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Abstract

Nitrogen use efficiency in crops is a primary concern in many parts of the world due to the low availability of nitrogen to plants. Microbial nitrogen fixation is a potential solution to this problem; however, the stability of nitrogen fixers in natural soil profiles is unpredictable. Also, hazardous soil elements like arsenic are a global threat to human health that is linked to soil-crop quality as well. Diazotrophic bacteria *Klebsiella oxytoca* can be used to confront these dual issues by implementing its ability to form biofilms. Using wild-type *K. oxytoca* M5a1 we have genetically modified it to express biofilm-developing genes (BssR and BssS), cyanase (CynS) and vacuolar arsenic sequestration gene (ArsR). These genes then allowed to better fix atmospheric nitrogen. The presence of a natural soil substance, cyanate, was also effectively used for additional ammonium production. The profuse development of biofilm allowed for a firm association with plant roots. These resulted in a higher translocation of available nitrogen from bacterial biofilm to the plant roots, detected using the N^{15} isotope in a secondary ion mass spectrometer. The biofilm also restricted the release of sequestered arsenic in vacuoles, as confirmed by differential cell elemental analysis. Plant vascular structures and root anatomy were closely monitored under these variable treatments and found to be structurally maintained compared to the control and stressed plants. Electron microscopy and energy-dispersive X-ray confirmed the structural rigidity and elemental distribution before and after the application of enhanced *K. oxytoca* M5a1, indicating its potential implementation in agro-environmental aspects.

From Sewers to Seas: microplastics as vectors for antimicrobial resistance from hospital wastewater to marine environments

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Abstract

Microplastics in the environment foster biofilms – the so-called 'Plastisphere'- but it remains inconclusive whether these communities may enrich antimicrobial resistant (AMR) or pathogenic bacteria. Whilst previous research has focussed largely on lab-based studies, *in situ* investigations are needed to clarify whether environmental selection or particle type primarily influence microbial community composition and AMR pathogen enrichment within microplastic-associated communities. This study evaluated *in situ* selective colonisation on microplastics (polyethylene, polypropylene and polystyrene) against natural (wood), inert (glass) and free-living (water) controls. Sterilised particles were attached to anchored, free-floating structures and incubated for two months across four different environments. These sites included a hospital wastewater tank, near to the effluent point of the wastewater treatment plant which receives both the hospital wastewater and domestic sewage, as well as downstream and offshore locations. Environmental metrics such as temperature, salinity and pH were also collected. Following incubation, biofilms and water samples underwent DNA extraction, with AMR gene presence assessed using 16S rRNA and *intI1* qPCR, followed by whole metagenome sequencing. Findings revealed that AMR gene enrichment varied by both substrate and environment, underscoring the unique role of microplastics in promoting AMR gene persistence in environmental communities across diverse settings. This work contributes to our understanding of how microplastics may support AMR development, persistence and dispersal in natural systems.

High-throughput enrichment and sequencing-based analysis of microplastic-associated microbial communities for the identification of plastic-degrading enzymes

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Abstract

Globally, over 10 million tonnes of plastic enters the ocean annually. In the environment, plastics abiotically fragment into smaller pieces, so-called microplastics, polluting soils, oceans and the atmosphere. The overall lifecycle and ultimate fate of microplastic in Nature is poorly understood, however, the recent discovery of microbial plastic-degrading enzymes indicate that microbes have the potential to bioremediate plastic. We aim to identify microplastic-associated microbial communities by growing them on plastic oligomers representing the two most common plastic building blocks: polyesters and polyolefins. To identify novel plastic-degrading microorganisms, we utilised microplate-based high-throughput assays to monitor microbial growth, redox-activity and esterase activity of microplastic-associated microbial communities from four environments (recycling centre, bay, tributary water and waste-water treatment plant) growing on two plastic oligomers: tetradecane and bis(2-ethylhexyl) terephthalate. We then used Illumina sequencing of 16S rRNA amplicons to understand the composition of the enriched microbial communities. Microplastic-associated microbial communities readily metabolised the plastic oligomers and showed elevated growth-rates, redox and esterase activities compared to controls without a substrate. Activities varied depending on the combination of substrate and inoculum. Over 3 million paired-end sequencing reads revealed the enriched microbial communities after 22 days of growth on each substrate. Our future work will involve enriching these microbial community on microplastics and using meta-omics analyses to elucidate plastic-degrading capabilities. Plastic-degrading enzyme candidates will be identified, expressed and tested for activity. Primarily, these enzymes will be applied for wastewater bioremediation within a novel wastewater-treatment technology developed by the BMRex consortium (www.bmrex-project.eu/) to minimise microplastic pollution.

Adaptions in an industrial *Streptomyces* lineage lead to increased antibiotic yield.

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Abstract

Random strain mutagenesis is used by producers of microbial natural products (MNPs) to increase yield. After exposure to mutagenic agents, the strains are screened and those showing desirable phenotypes are taken forward. However, unlike targeted improvements, the genetic causes of these mutations are unknown and can therefore not be replicated in other MNP producers. Such a process was used by GSK to evolve strains of *Streptomyces clavuligerus* that are able to produce increased yields of clavulanic acid during fermentations. A process which generates over £500 million a year for GSK.

GSK has provided the first six strains of their random mutagenesis lineage for phenotypic, genomic and transcriptomic analysis. We have discovered a high degree of genome plasticity showing both large scale macro genomic rearrangements and 56 single nucleotide changes. The strains have gradually lost metabolic flexibility as they are more specialised for fermenter conditions and media. This presents a problem for any attempts to change the fermentation process, for example to switch to more sustainable feedstock.

However, under GSKs fermentation conditions the later lineage strains produce a significantly higher yield of clavulanic acid. We have selected 25 of the SNC found in later lineage strains and have produced a series of overexpression vectors which have been complemented back into the progenitor strain. We will be using this library to identify key mutations which result in increased clavulanic acid yield and that reduce metabolic flexibility. These will then be tested in other antibiotic producing *Streptomyces spp.*.

Engineering algal phycosphere for bio-remediation, bio-polymer production, and novel material development.

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Abstract

Harnessing the interactions between microalgae-bacteria provides a promising solution for multiple applications, including bioremediation and biomanufacturing. This study demonstrates co-culturing of bacteria with alga *Nannochloropsis oculata* as artificial 'phycosphere' consortia to achieve bioremediation of industrial leachate, production of third-generation bioplastics, polyhydroxyalkanoates (PHA), and microalgal cellulose as novel eco-friendly materials. *N.oculata* showed fast growth in 100% industrial leachate adjusted to marine salinity (3.3%). However, diluted leachate (20%-50%) and 40 µmol/m²/s of light intensity was preferable for balancing light delivery and growth rate. A collection of environmentally isolated bacterial strains were selected for PHA producing potential via Nile Red staining, *phaC* gene PCR and GC-FID analysis. Bacterial combinations were also screened for compatibility with *N.oculata* in co-cultures to identify supportive strains. Strains from genera *Microbacterium*, *Rhodococcus*, *Sphingobium*, and *Brevundimonas* enhanced *N. oculata* growth in leachate, yielding approximately 2.5% PHA, characterised by both scl- and mcl-polymers and up to 14% cellulose content (w/w dry biomass). Transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM) imaging of purified cellulose revealed a unique spherical "microcage" morphology with high crystallinity and structural integrity, suggesting its potential as a robust novel material. Certain bacteria also altered algal cell wall thickness, and whole-genome sequencing of these bacterial strains is ongoing to identify genes and molecules associated with observed interactions. The optimal consortium will be tested in a 200L bioreactor, monitoring leachate composition (COD, nitrogen, phosphorus) to evaluate bioremediation and waste-to-product conversion. Species-specific qPCR and FISH will reveal consortia community structure dynamics.

Biocoatings: Confining genetically modified *Escherichia coli* for the production of hydrogen

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Abstract

A biocoating is a waterborne colloidal coating containing live bacteria. They have the potential to overcome many of the limitations presented by traditional bioreactors, including contamination and transport of bacteria. Here, genetically modified *Escherichia coli* were confined within a biocoating for the purpose of hydrogen production. To manufacture the biocoatings, a gelation method was used, wherein salt induces the coagulation of the colloidal particles, thereby avoiding water evaporation and reducing osmotic stress. This method significantly increased the bacterial viability compared to the conventional desiccation method. When biocoatings were fully formed in media and never dried, the osmotic pressure is reduced even further, thus increasing cell viability.

Biohydrogen is an attractive alternative to more traditional hydrogen production methods because it does not require fossil fuel feedstocks. Double knockouts of genes involved in the mixed acid fermentation pathway were found to increase the hydrogen yield, overcoming one of the key limitations of biohydrogen for large-scale production. The best-performing double gene knockout *E. coli* were confined within biocoatings, and their hydrogen production measured using microsensors. The best-performing strain had a hydrogen production rate that was 100 times higher than the wildtype strain. Interestingly, the production rate was higher than obtained from bacteria in liquid suspension, suggesting a positive impact of cell confinement. The production rate of carbon dioxide (a greenhouse gas) was also significantly lower than for the wildtype, making this method of hydrogen production an attractive alternative to grey and brown hydrogen methods.

Designing sustainable co-cultures for biomanufacturing: Exploring the effects of spatial configuration on metabolism and interactions in a synthetic microbial consortium using quantitative proteomics

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Abstract

Microorganisms naturally exist within complex communities, which exhibit incredible metabolic versatility and robustness. Inspired by these natural systems, synthetic microbial consortia have been developed to enhance the production of valuable biochemicals. In this study, we constructed a synthetic microbial consortium comprising two genetically engineered strains: *Synechococcus elongatus* PCC 7942 cscB/SPS, a freshwater cyanobacterium that fixes atmospheric carbon and secretes sucrose, and *Azotobacter vinelandii* AV3, a nitrogen-fixing soil bacterium that secretes ammonia. These organisms engage in a sustainable cross-feeding relationship, supplying each another with bioavailable nitrogen and carbon. However, our growth data suggests the microorganisms may undergo competition and stress when grown together.

To explore the optimal cultivation conditions for our synthetic consortium, we designed a bioreactor system that enabled us to compare two distinct spatial configurations: a 'mixed' condition, where the species were grown together with direct cell-to-cell contact and nutrient exchange, and a 'separated' condition, where the organisms were physically partitioned by a central membrane, facilitating metabolite exchange without direct interaction.

We used quantitative label-free proteomics to characterise the metabolisms and interactions between the two species under each spatial configuration, facilitating the generation of testable hypotheses for optimising co-culture conditions. Insights gained from improving the stability of this system will inform the design of more complex microbial consortia for biotechnological applications, such as modular systems where additional microbes can be introduced to produce valuable chemicals. Our findings also offer general considerations for designing more robust and productive synthetic microbial consortia.

Balancing the scales: assessing the impact of irrigation and pathogen burden on potato blackleg disease and soil microbial communities

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Abstract

Understanding how environmental factors, crop yields, and soil health interact is essential for sustainable agriculture under changing climate conditions. In potato production, irrigation management can increase blackleg disease risk, while drier conditions favor common scab. The specific mechanisms connecting these factors to soil microbiome dynamics are not well understood. This study examined how irrigation practices and pathogen load in seed stocks impact (i) crop yields, (ii) disease development (blackleg or common scab), and (iii) soil microbial community dynamics.

We grew seed potatoes with varying *Pectobacterium* levels (Jelly [high load], Jelly [low load], and Estima [zero load]) under four distinct irrigation regimes. Soil microbial communities were analysed using amplicon sequencing of the 16S rRNA gene using DNA extracted from soil at 50% emergence and at harvest. Using generalized linear latent variable models and ensemble quotient analysis, we explored interactions between irrigation regimes, pathogen levels, and microbial communities.

Results showed that irrigation increased blackleg incidence in seed stocks with low and high *Pectobacterium* loads (22–34%) but not in zero stocks (2–6%). Withholding irrigation raised common scab occurrence (2–5%) and decreased crop yields. While irrigation did not change soil microbiome composition, high *Pectobacterium* loads in seed stocks increased the abundance of Planctomycetota, Anaerolinea, and Acidobacteria. Ensemble quotient analysis revealed the *Anaerolinea* taxa were strongly associated with high levels of *Pectobacterium*in in the seed stock and blackleg symptoms in the field.

Our findings suggest that managing pathogen loads in seed stocks significantly influences soil microbial communities, impacting crop health and productivity.

The role of environmental amoebae in the survival of bacteria and the spread of AMR

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Abstract

Understanding the role that environmental conditions play in the emergence of resistance in bacteria is crucial in tackling the antimicrobial resistance (AMR) crisis. An interdisciplinary approach was used to determine key physicochemical drivers of resistance within the environment and to evaluate the role of host organisms such as amoebae. Currently AMR is predicted to cause an extra 14.5 million deaths annually by 2050.

Key geochemical parameters such as pH, conductivity, Total Dissolved Solids (TDS), Organic Matter (OM) were determined. Potentially toxic elements (PTEs) were quantified using Inductively coupled plasma mass-spectrometry and atomic-emission-spectrometry (ICP-MS/AES).

Environmental amoebae were isolated and identified using Sanger Sequencing. Bacteria isolated from extra- and intra-cellular matrices were tested for resistance to key clinical antibiotics using antimicrobial susceptibility testing (ASTs). In addition, resistance to key anthropogenic PTEs; As, Cd, Cr, Cu Hg, Pb, Ni, Zn; was testing using an adapted Minimum inhibitory concentration (MIC) to quantify bacterial susceptibility using absorbance to measure bacterial growth.

The intrinsic link between environmental amoebae and bacteria demonstrates an evolutionary survival mechanism which is shown in the differences between extra- and intra-cellular microbial communities and levels of resistance that they harbour. Harsh environments such as volcanic soils sites offer an insight to how microbial communities shift in response to physicochemical properties and contaminants in the environment. Without fully understand these environmental influences on resistance, the prevalence of AMR will continue increase and will ultimately impact global One-Health.

Exploring Hidden Threats: Knowledge Expansion of Understudied Bacterial Pathogens

Invited: *Burkholderia* bacteria: expanding our knowledge of their taxonomy and pathogenesis using genomics

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Abstract

Burkholderia bacteria are a diverse genera of gram negative bacteria. As pathogens, they are most well-known for causing lung infections in people with cystic fibrosis (pwCF). Contamination of sterile pharmaceutical and other industrial products with *Burkholderia* has also been linked outbreaks of opportunistic human infection. They are also associated food-poisoning due to the production of the toxin bongkrekic acid and cause various diseases in range of important crop plants. As CF lung pathogens, a closely related group of species known as the *Burkholderia cepacia* complex emerged as transmissible infections in the 1990s. They were difficult to identify using conventional microbiology methods, but the application of genomics has now revealed more than 25 species groups withing the *B. cepacia* complex. Of these, *Burkholderia multivorans*, *Burkholderia cenocepacia*, and *Burkholderia oribicola* are the most prevalent *Burkholderia* lung infections seen in pwCF. Another *Burkholderia* species, *Burkholderia gladioli*, is also able to cause CF lung infections, and in agriculture this species is problematic cause of onion and mushroom rot. Evolutionary sub-groups within *B. gladioli* that encode the toxin bongkrekic acid can cause lethal food-poisoning when ingested in range of fermented food and drinks. Overall, *Burkholderia* bacteria are true opportunists in the context of pathogenesis, being frequently associated with beneficial interactions with plants and other organisms, but when hosts become vulnerable they switch to disease-causing pathogens.

Invited: Diphtheria agents revisited through the lens of genomics

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Abstract

Diphtheria, “the strangling Angel of children”, is a severe respiratory infection with a high case fatality ratio in unvaccinated, untreated populations. Although largely controlled by vaccination, diphtheria is currently re-emerging, and large outbreaks occurred in recent years.

The classical agent of diphtheria, *Corynebacterium diphtheriae* has been a pioneering model for the microbiology of pathogenesis (discovery of toxins), for vaccination (invention of toxoid vaccines), and for the evolutionary dynamics of virulence (discovery of corynephages as vectors of diphtheria toxin). But given the remarkable success of vaccination, microbiologists have almost entirely forgotten this pathogen, largely understudied in the last decades. The reemergence of diphtheria now stimulates renewed interest in the epidemiology, genomics and pathogenesis of this historically prominent disease.

I will present recent taxonomic updates and research on the population genomics of *C. diphtheriae*, revisiting the classical microbiology of diphtheria and defining the diversity of species and strains that may cause diphtheria. The evolutionary links between toxigenic and non-toxigenic strains, highly relevant for epidemiological surveillance and outbreak investigations, will be presented, revealing the diversity and dynamics of the prophages that carry the *tox* gene. I will also present recent discoveries on the molecular basis of antibiotic resistance and will illustrate how the use of genomics can help tracing spatial dissemination of diphtheria strains from local to international scales.

Recent advances in the genomics of diphtheria are improving our collective ability to understand and control the disease, but need to be implemented more widely, using common genotyping standards and data sharing platforms.

Creating a molecular epidemiology framework for *Mycobacterium leprae*

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Abstract

Leprosy is a chronic infection of the peripheral nerves, causing numbness, muscle weakness, and skin lesions. Each year, 200,000 new cases are reported, primarily concentrated in a few high burden countries. Global eradication of leprosy relies on the detection of transmission clusters to inform targeted interventions. *Mycobacterium leprae* is the primary pathogen causing leprosy. Molecular epidemiology of M. leprae is in its infancy as this bacterium is unculturable, hindering whole genome sequencing efforts. Recent advances in DNA extraction and sequencing have overcome some of these challenges, creating a need for a robust molecular epidemiology framework to enable detection of transmission clusters based on sequence variation.

This project aims to address this challenge by estimating the effectiveness of both a reference-based mapping approach (using the Snippy pipeline) and a reference-free distance estimation (using SKA-2) to approximate the SNP distances between clinical isolates. We use a set of four complete genomes to first estimate the precision of each approach for estimating SNP distances compared to a ground truth estimated by Mauve whole genome alignments. We find that both approaches have high accuracy for estimating SNP distances, despite the exceptionally low diversity observed in *M. leprae*. We then extend this approach to a large set of clinical isolates to better understand how these SNP distances may translate into transmission cluster reconstruction.

Our findings indicate that a robust clinical pipeline for calling SNPs and reconstructing transmission clusters is possible for M. leprae, allowing for molecular epidemiology approaches to be incorporated into leprosy control efforts.

Helicobacter suis in UK farmed pigs and retail meat products: implications for zoonotic transmission

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Abstract

Helicobacter suis primarily colonises the stomach of pigs, causing diseases similar to the highly virulent human-adapted *Helicobacter pylori*, raising significant animal welfare concerns. In humans, *H. suis* is the predominant non-*H. pylori Helicobacter* species found in gastric biopsies and is associated with conditions such as gastritis, gastric ulcers, MALT lymphoma, and increasingly found in patients with gastric cancer. Contaminated food products are thought to be a potential source of transmission between pigs and humans. However, research supporting this is limited, and the presence of *H. suis* in food products in the UK remains unstudied, with no reported culture or prevalence rates.

This study aimed to assess the contamination of raw pork products for sale in the UK with *H. suis*, employing a culture-based methodology to identify viable bacteria. Additionally, the prevalence of *H. suis* infection in pigs was investigated by collecting stomach samples post-slaughter. Molecular techniques, histology, and long-read sequencing were employed to confirm *H. suis* isolation, supporting additional genomics analysis.

Viable *H. suis* was successfully cultured from 4 out of 95 (4.2%) meat samples and from 6 out of 7 (85.7%) pig stomachs. These findings suggest that *H. suis* contamination is present in UK raw pork products and indicate a high prevalence of the infection in UK farmed pigs. This study also reports the first UK *H. suis* isolate genomes.

Genetic Variants in Yaws Linked to Antibody Responses and Persistence

Amber Barton[1], Petra Pospíšilová[2], Camila G Beiras[3,4], Lucy John[5], Wendy Houinei[5], Lorenzo Giacani[6,7], David Šmajs[2], Michael Marks[8,9,10], Oriol Mitjà[3,4], Mathew Beale[1], Nicholas Thomson[1,8]

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Abstract

Yaws, a neglected tropical disease caused by *Treponema pallidum* subspecies *pertenue*, predominantly affects children in rural communities in the Pacific and Africa. While initial infection causes ulcers that heal in 3-6 months, the bacteria can lay dormant in the body for years before causing secondary lesions and disabling bone damage. As *T. pertenue* has never been successfully cultured *in vitro*, it has not been possible to isolate for sequencing or perform controlled *in vitro* experiments, limiting our understanding of its biology.

Here we carried out bait-capture and sequencing on 263 PCR-positive swabs from a trial investigating repeated mass drug administration as a means of yaws elimination. We successfully recovered 222 good-quality *T. pertenue* genomes, and identified 44 genes containing variants associated with decreased treponemal antibody (Mann-Whitney test, adjusted p<0·05), amongst which proteins with signal peptides were over-represented (p = 0·0008, Fisher's test), suggesting that secreted and membrane proteins are involved in immune evasion.

We also found that yaws sub-lineages which persisted after repeated mass drug administration had mutations in penicillin-binding proteins: I415F in *pbp2* and A506T in *mrcA*. These variants both previously independently arose in *T. pallidum*, the causative agent of syphilis, suggesting they confer a selective advantage. We hypothesise that mutations in penicillin-binding proteins affect cell envelope biogenesis, reducing their growth rate and therefore interfering with immune activation and bacteriostatic antibiotics. These observations illustrate the value of bait-capture and whole-genome-sequencing to investigate the biology of unculturable pathogens.

Exploring hidden threats: expanding our knowledge of the antimicrobial resistance transmission risks of as yet unculturable anaerobic bacteria.

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Abstract

The growing concern of antimicrobial resistance (AMR) within livestock poses significant risks to animal and human health through the potential transmission of resistant bacteria and genes. Exploring the hidden threat of AMR in uncultured rumen bacteria and understanding the distribution and dissemination of resistant genes within the rumen environment is critical for mitigating One Health risks. In this study, we investigated the AMR distribution and transmission risks in ruminal pure culture microbial genomes and metagenomic genomes collected from JGI and NCBI using PanRes and MobileOG. Biocide/metal and tetracycline antimicrobial resistant genes (ARGs) dominated in the rumen pure culture dataset while glycopeptide ARGs were more prevalent across the MAGs. Eight uncultured taxonomic classes including *Anaerolineae, Vampirovibrionia, Xenobia, Methnobacteria, Spirochaetia, UBA1177, Kiritimatiellae* and *Lentisphaeria* were found to harbour ARGs in the aminoglycoside, fluoroquinolone, glycopeptide, polymyxin, streptogramin A and tetracycline classes, which indicates worthwhile gaps for studying unculturable microbes. ARGs were detected in 50 out of a total of 72 uncharacterised rumen microbial genomes. Moreover, 38 putative mobile genetic elements were found carrying at least one ARG in 20 ruminal pure culture bacteria genomes, including multidrug-resistant genes in *Fibrobacter sp.* UWB12 flanked by a pair of *IS612*. Work to validate the potential transmissibility of these ARGs in the newly isolated ruminal strains *in vitro* is ongoing. Our findings offer valuable insights into potential hidden threats presented by unculturable microbial populations in complex ecosystems with broader implications for the AMR challenge and solutions for mitigating AMR proliferation in the One Health world.

Zoonotic infection in *Streptococcus suis* strongly associates with specific capsule diversity

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Abstract

Capsule polysaccharides are the outmost structures of many pathogenic bacteria and play a crucial role in host-pathogen interactions. The composition of capsule usually determines the pathogen's serotype. *Streptococcus suis* is an important swine pathogen and zoonotic agent, capable of causing human infections. The majority of human *S. suis* cases are caused by isolates from a specific genetic lineage (clonal complex (CC) 1) with capsule serotype 2 or 14. However, studies on the diversity of *S. suis* capsules and the relationship between capsular switching and human adaptation remain limited. We analyse 6,439 *S. suis* genomes (including 965 human isolates), determining the serotype and CC of each isolate *in silico*. In this dataset we identify 61 evolutionary independent human-associated capsular switching events, where human infections are associated with a different capsule than the most closely-related pig isolate. We found 58/61 of these events switched to serotype 2, two to serotype 5, and one to serotype 14. By extracting all capsule gene clusters and constructing a protein-sharing network, we found that the serotype 2 capsules clustered into three subtypes. Subtype-I was the most prevalent and closely related to CC1 isolates, while subtypes-II and III were more divergent. Notably, 42 out of the 58 serotype 2 human-associated capsular switching events switched to subtype-I. Our results imply a subtype I serotype 2 capsule is the main determining factor in facilitating *S. suis* human infection, which has implications for preventing and treating zoonotic infections.

A28Resolving 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]

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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.

From Microbes to Meals

Invited: Reimagining Chlorella as a food and beverage ingredient suited for everyday foods

Andrew Spicer, Emma Ransom-Jones, Gino Schiano Di Visconte, Aaron Dickson, Luke Townsend, Suzy Smith

Algenuity, Stewartby, United Kingdom

Abstract

Freshwater microalgae of the genus Chlorella and close relatives have long been recognised as nutritional powerhouses, with biochemical compositions rich in complete protein, dietary fibre, vitamins and minerals while being comparatively low in saturated fats. The high chlorophyll level (2 to 3% dry weight) typical of Chlorella grown outdoors as well as the poor product consistency and quality have limited the application of native Chlorella biomass in everyday foods; simply put, taste is king and green, bitter-tasting foods don't sell well.

In order to transform the nutritional opportunity represented by Chlorella, we have applied mutation plant-breeding (chemical mutagenesis) and careful selection plus aligned bioprocess and downstream processes to derive strains of both Chlorella vulgaris and Chlorella sorokiniana that yield whole-cell biomass ingredients improved for colour, with white strains being the most highly desirable, relative protein content, relative digestibility and productivity as well as sensory quality (flavour).

We have determined full annotated genomic sequences for approximately 100 derived, improved strains and demonstrated defined technoeconomic models that have been validated at commercial scale through high productivity microbial fermentation.

Furthermore, we have derived full Life Cycle Assessment representing the end to end process.

Improved Chlorella can be utilised to replace eggs and/or dairy in a broad range of everyday foods at replacement levels as high as 100% with no compromise in mouth-feel or food enjoyment.

These ingredients are allergen free, and not novel with regard to food regulations. I'll present the science and real world applications that will support the food transition.

Invited: Mycoprotein as a scalable and sustainable solution to global food challenges

Katarzyna Polanowska ORCID iD

ENOUGH, Sas van Gent, Netherlands. Poznań University of Life Sciences, Poznań, Poland

Abstract

Mycoprotein is a protein-rich fungal biomass with a reduced RNA content, typically derived from *Fusarium venenatum*. It serves as a sustainable and versatile ingredient in alternative protein production. Enough, a leading food technology company, specialises in producing mycoprotein using a patented submerged fermentation process, which is designed to minimise waste and maximise efficiency.

During its scale-up journey, Enough secured significant funding to advance its research and development initiatives. This includes the €17m Plenitude project, funded by the Circular Bio-based Europe Joint Undertaking (CBE JU), provided comprehensive opportunities to study mycoprotein's characteristics and applications. This research with Wageningen focused on key aspects such as texture-creation properties, functional applications, digestibility, shelf life, and sensory attributes. Additionally, project partners explored innovative uses for mycoprotein, including its potential role in producing biodegradable bioplastics and its integration into hybrid products combining cultured animal cells with fungal-based components.

These advancements underline Enough’s commitment to driving sustainable food innovation while addressing global challenges in protein supply and waste reduction.

Invited: Exploring intra-Species diversity in fungi for novel Mycoprotein production

Chloe Uyil, Michaela Kolmosova, Robert Barker, Alessia Buscaino

University of Kent, Canterbury, United Kingdom

Abstract

Fungal organisms have played a fundamental role in the food and drink sector for millennia, contributing to the production of bread, beer, and a variety of fermented products. More recently, fungi have been successfully used for the development of mycoprotein, a sustainable and nutritious alternative to animal-based food products. This innovation has provided a promising solution to the growing demand for protein sources with lower environmental impact.

Fungi are incredibly diverse, exhibiting a wide range of growth capabilities, morphologies, and nutritional profiles. This variation exists not only between different fungal species but, remarkably, also among isolates of the same species. Such intra-species diversity represents an untapped potential for optimising and expanding the use of fungi in food production.

In this presentation, I will outline our ongoing research efforts to explore and exploit this diversity to develop novel fungal platforms for mycoprotein production.

Evaluation of the microbial risk associated with duckweed use for feed applications following cultivation on farmyard wastewater.

Leah Bannon[1], Cian Redmond[2], Marcel Jansen[2,3], Niall O'Leary[1,3]

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Abstract

In Europe, the livestock sector is one of the biggest consumers of plant protein, with 70% being directed to pig and poultry feed alone. It is a challenge for the sector to meet this demand, resulting in a reliance on imported soy protein in several countries. Duckweed species are free-floating freshwater plants belonging to the family Lemnaceae. These plants can be found worldwide and are characterized by high growth rates and high protein content (up to 35% per unit dry weight). In addition, these plants have been shown to grow on a range of high nutrient load, agricultural and municipal waste streams. Duckweed species therefore offer sustainable, circular economy opportunities to couple wastewater valorisation with high protein content plant growth for potential animal feed applications. However, a critical aspect of any such process would be to evaluate the potential microbiological risk of pathogenic bacterial associations with wastewater grown Duckweed.

In this study, duckweed was cultivated on farmyard wastewater (dairy milking parlour and yard waste) over a 28-day period, after which wastewater and frond-associated bacteria were isolated. The dominant genera recovered from Duckweed contained several plant growth promoting bacteria, namely Pseudomonas, Comamonas and, Herbaspirillum. These genera have previously been identified as common community members of duckweed associated microbiomes. Notably, no enteric bacterial isolates were recovered from fronds, however Aeromonas salmonicida, a known fish pathogen species was found in all samples.

Significance of the findings and ongoing investigations with abattoir wastewaters are discussed.

Genetics and Genomics Forum

Advances in Cataloguing Bacterial Population Structure: Development of a Life Identification Number (LIN) Barcoding System for Neisseria meningitidis

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Abstract

Accurate prokaryotic taxonomy is key for understanding population structure and dynamics. Life Identification Number (LIN) barcodes are a novel way of describing bacterial species through stable hierarchical clustering. These are based on allelic differences between core genome Sequence Types (cgSTs), assigned from representative core genome MLST (cgMLST) profiles. *Neisseria meningitidis* cgMLST v3 (1,329 loci), available on PubMLST, was used as the basis of this work.

A curated dataset of 6,131 *N. meningitidis* isolates, encompassing up to 200 chosen isolates from each clonal complex (CC), was used for LIN code development. The cgSTs for each isolate were used to generate a pairwise distance matrix and analyses using Minimum Spanning Tree-based clustering. Overall, 14 LIN thresholds were chosen to represent different genetic lineages. These will be assigned human-readable nicknames that are consistent with existing historic MLST nomenclature. Defined *N. meningitidis* LIN thresholds are openly available for use on PubMLST.

Several published outbreak datasets were used to validate the LIN code systems, which illustrated high-quality and fine resolution for population analyses. In addition, it was possible to differentiate the Hajj and South American CC-11 meningococcal variants at the LIN threshold of 5.04% allelic mismatches. This mismatch threshold represents clonal group, illustrating very similar bacterial variants. Overall, LIN codes will be important for distinguishing closely related variants in outbreak investigations, contributing to our understanding of strain theory, and aiding vaccine development.

Antimicrobial resistance mechanisms and the investigation of novel approaches to control infection by the human pathobiont *Fusobacterium nucleatum*

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Abstract

Background:

Fusobacterium nucleatum is a human pathobiont of the oral microbiome with a key role in the progression of periodontal diseases. In recent years, the species has been linked to numerous systemic diseases, including colorectal cancer, adverse pregnancy outcomes, irritable bowel disease and endometriosis. The aim of this research is to examine type strains and novel oral isolates for sensitivity to conventional and novel antimicrobial strategies.

Methods:

Whole genome sequencing of type strains and novel oral isolates of *F. nucleatum* was completed using Oxford Nanopore long read sequencing and annotated with Bakta. Antibiotic target genes were extracted from the annotated genomes and compared with sensitivity to a panel of antimicrobials, including ampicillin, metronidazole and novel agents. The value of adjuncts was also explored.

Results:

Variation was observed across strains for resistance to ampicillin. As a single agent, metformin did not exhibit any antifusobacterial activity, however, under laboratory conditions, metformin potentiated the effect of vancomycin in some strains, where vancomycin alone had no effect. Standard tools to identifying AMR genes did not identify any genes in any *F. nucleatum* strains. Manual searches did reveal a difference in the carriage of beta-lactamases and key targets including cell wall synthesising enzymes.

Conclusion:

This research highlights the significant genetic diversity that exists in the species *F. nucleatum.* Our work will form the basis for development of novel strategies to deal with an increasingly important human pathobiont and is intended to result in targeted antimicrobial approaches in relation to microbiome associated human disease.

CRISPR/Cas9 gene editing facilitates antibiotic discovery in Streptomyces formicae KY5

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Abstract

Actinomycetes like *Streptomyces* are known to encode numerous biosynthetic gene clusters (BGCs) that make clinically relevant antimicrobial molecules. Unfortunately, most of these BGCs are cryptic under standard laboratory conditions, therefore the pathways and the resulting molecules remain uncharacterised. *S. formicae* KY5 was isolated from the nest of *Tetraponera* ants and is known to produce the formicamycins, potent antimicrobials with activity against drug resistant pathogens like MRSA. Analysis of the *S. formicae* genome shows the strain encodes as many as 45 secondary metabolite BGCs, most of which look novel, but only the formicamycin BGC is so far characterised. In this work, we show that deletion of the formicamycin BGC using CRISPR/Cas9 induces the expression of a previously cryptic NRPS BGC in *S. formicae*. The product of this BGC is a potent inhibitor of gram-positive pathogens including MRSA and is readily isolated under standard laboratory conditions. Using genetics and comparative metabolomics we have further characterised this novel biosynthetic pathway and are making efforts to elucidate the mode of action of the product. We are also aiming to understand why deletion of the formicamycin BGC activates expression of this NRPS with a view to developing genetic tools that can be applied more widely to other talented strains. This work shows the importance of genetic rewiring as a tool for antibiotic discovery, and highlights the complex interplay between different secondary metabolite pathways present within the same strain.

Location, location, location – how does genomic context affect gene expression?

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Abstract

Regulated gene expression that produces the appropriate complement of transcripts for survival in a given environment is critical for all organisms. Whilst much of the control of gene expression is governed by local factors that impact transcription rates, genome position and chromosome organisation are also important influences on gene expression. This is recognised in eukaryotes, but similar processes remain poorly understood in bacteria. We do know that within bacteria, all genomic locations are not equally suitable for transcription of a given promoter, and the micro and macro structures of bacterial chromosomes are dynamic. In combination, these factors are major influences on transcription.

Here, we present an optimised method to study how genomic context influences gene expression in bacteria and explore the effect of environmental stresses on this relationship. To identify differences in expression from the same transcriptional unit across the genome, a method of sequencing RNA from massive libraries of *Escherichia coli* and *Salmonella* Typhimurium transposon mutants was developed. Measurements of transcription, from the identical outward-facing inducible promoter within each transposon, allowed the impact of changes in the environment on expression to be determined and correlated with precise measurements of chromosome supercoiling. This approach provides a high-fidelity method that can be used to study the interdependency of gene expression, gene location and DNA topology. Developing an improved understanding of the relationship between genome structure and the regulation of gene expression will provide insight into genome evolution and information that can be applied in synthetic biology.

Going beyond resistance genes: detection of genomic co-adaption to resistance determinants

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Abstract

Decreasing efficacy of antibiotics due to increasing bacterial resistance threatens modern medicine. Mitigating this escalating problem depends upon detailed understanding of how the genome accommodates genotypic changes that occur on the pathway to resistance. Bacterial genome-wide association studies (GWAS) transformed the identification of the genetic basis of resistance in several pathogens. But now work must be done to describe the events that precede and succeed resistance in the evolutionary timeline. Here we present a novel technique 'masking GWAS'. This extends the GWAS approach to go beyond phenotype-genotype and detect genotype-genotype relationships via genomic masking. The usability of this method is demonstrated in two species: *Staphylococcus aureus* and *Escherichia coli.* We analyse over 9,000 genomes to identify covarying genes that are linked to the acquisition of clinically relevant resistance determinants (SCC*mec, blaCTX-M, blaOXA, mcr*). We show that signatures of selection vary between species, determinants, and phylogroups of *E. coli*. Accurate detection of potentiating and compensatory genomic changes due to acquisition of resistance determinants is vital for understanding antimicrobial resistance and the nature of emergence of multi-drug resistance.

How do we translate genomics into deployable tools for high-resolution pathogen surveillance in low-resource settings?

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Abstract

Introduction: Genomic pathogen surveillance has increasingly become a useful technique for research and public health. Discriminating closely related strains and tracking populations of pathogens can enable understanding of population structures, as well as providing insights into epidemiology, transmission and the impact of clinical interventions. However, sequencing whole pathogen genomes (WGS) at scale remains unaffordable or impossible in low-resource settings. Current alternatives to WGS, such as multi-locus sequence typing (MLST) offer comparatively low resolution and fail to distinguish key lineages in important human pathogens. Moreover, both WGS and MLST are costly, with bacterial genome sequencing costing >£65 commercially in the UK.

Methods: We developed a phylogenetically informed SNP typing approach which maximises phylogenetic discrimination whilst minimising the number of target regions, enabling reconstruction of fine-scale sublineages. Using *Treponema pallidum* (causative agent of syphilis) as a model, we designed and validated a high-resolution multiplex PCR scheme comprising 59 discrete genomic regions which were sequenced using MinION Flongle Cells. We then developed an end-to-end bioinformatics pipeline in NextFlow and a web application for easy interpretation.

Results: Using 72 clinical samples from South Africa, the assay was comparable in accuracy and reproducibility to Illumina WGS, enabling fine-scale delineation of recent genomic clusters at a cost of <£10/sample. We deployed our method in a low resource laboratory in Zimbabwe, successfully detecting and profiling 17 *T. pallidum* samples.

Discussion: This method has broad applications for enabling scalable genomic surveillance across a range of pathogens, and we are developing further schemes for field deployment.

Exploring de Bruijn Graphs as a Framework for Metagenomic Data Representation

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Abstract

Metagenomic analysis is a crucial approach for elucidating microbial community structure and dynamics, with significant implications for applications such as pathogen surveillance and ecological monitoring. Traditional genome-resolved methodologies often prove inadequate when addressing fragmented, complex metagenomic datasets, particularly those involving uncharacterised genomes. This study investigates the utility of de Bruijn graphs (DBGs) as a scalable, reference-free approach for representing metagenomic data, focusing on their capacity to capture genomic diversity and structural features across varying coverage levels, sequencing error profiles, and genome similarities.

Simulated multi-species metagenomic samples were generated using the Metagenomic Sequence Simulator (MeSS) and subsequently analysed with MetaGraph to construct primary compacted DBGs, incorporating k-mer frequency-based graph cleaning methods. Preliminary analyses reveal that high-coverage genomes produce longer, less fragmented contigs, while low-coverage genomes result in substantial fragmentation, compromising the quality of the resulting DBG. The presence of sequencing errors exacerbates connectivity issues, introducing spurious components and reducing overall mappability. Additionally, taxonomic resolution can be hindered by genomic similarity, as observed in closely related taxa such as *Haemophilus influenzae* and *H. parainfluenzae*.

Future research will aim to optimise k-mer lengths to enhance graph connectivity for low-coverage genomes, explore advanced error correction strategies to mitigate the influence of sequencing errors, and employ network algorithms for more precise delineation of taxonomic boundaries within large, interconnected components. Ultimately, this research seeks to refine DBG-based methods to improve scalability and accuracy, validating these approaches on real-world metagenomic samples to develop a robust framework capable of handling diverse microbial communities and complex metagenomic datasets.

Genomic rearrangement of capsule operon in multi-drug resistant *Escherichia coli* lineage

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Abstract

Capsule is a known virulence factor in pathogenic *Escherichia coli*, contributing to immune evasion and host colonisation. Over 80 serologically-unique capsule types have been identified, categorised into four groups depending on biochemical and genetic properties. Here, we investigated gene variation in group 2 capsule types, associated with extra-intestinal infection. We focused specifically on *kpsM* and *kpsT* which encode proteins that facilitate polysaccharide transport across the inner membrane, aiming to understand the evolutionary pressure on these conserved genes.

The presence and allelic variation of *kpsM* and *kpsT* was assessed across a phylogenetic tree of 3033 *E. coli* ST131 genomes, a pandemic multi-drug resistant (MDR) lineage. Distinct subclades were then examined, and long read sequencing performed on a selection of isolates with uncommon nucleotide sequences to assess the genomic arrangement.

An unusual variant was identified with only 67% nucleotide identity to the other *kpsM* genes in this lineage but 96% amino acid identity. This allele was not only rare in our dataset, confined to a single clone, but could not be found outside ST131 clade C following a wider BLAST search. Long-read sequencing revealed a structural arrangement different to any of the reported capsule groups.

The rarity and genomic arrangement of this allele suggests it may have arisen due to recombination, although the similarity at the protein level indicate these genes may still be functional. The dramatic rearrangement in a key virulence factor may have important ramifications for the success of this MDR lineage.

Can shared antibiotic use promote host jumps? Evidence from zoonotic *Streptococcus suis* in birds

Muriel Dresen ORCID iD[1,2,3], Gemma G. R. Murray[4], Peter Valentin-Weigand[3], Marcus Fulde[2], Lucy A. Weinert[1]

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Abstract

The interconnectedness of human, animal, and environmental health is a challenge for emerging health threats, such as antimicrobial resistant bacteria. For example, widespread use of similar antimicrobials in both human and livestock may play a role in interspecies bacterial transmission by disrupting natural microbial communities and creating a common survival environment that favours resistant bacteria**.** Pigs and poultry receive the highest levels of antimicrobials and consequently frequently harbour multidrug resistant bacteria. One ubiquitous pig pathogen, *Streptococcus suis* that also spills over to cause disease in humans, commonly exhibits frequent multidrug resistance. Here we show from a sample of over 3000 *S. suis* isolates from pigs, wild boar, humans, cats, dogs, cattle, and birds that a multidrug resistant lineage, distinct from the lineage responsible for most zoonoses, shows signatures of adaptation to birds. We find long-term phylogenetic persistence in poultry populations, with multiple host jump events and subsequent transmission to wild and pet birds. Moreover, we identified unique mobile genomic islands and a trend for greater survival in chicken versus pig blood. While chickens may not be a primary source of zoonotic *S. suis* infections, our results suggest that shared antibiotic usage in pigs and poultry may have promoted a host jump of these antimicrobial-resistant bacteria. Increasing intensification of livestock production may enhance transmission of antimicrobial bacteria to other animals or humans, emphasising the need of a One Health approach.

Convergence and global molecular epidemiology of *Klebsiella pneumoniae* plasmids harbouring the *iuc*3 virulence locus: a population genomic analysis.

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Abstract

Klebsiella pneumoniae (Kp) is an important pathogen of humans and animals. The plasmid-borne *iuc* locus, encoding the siderophore aerobactin, is a key virulence factor in Kp; the variant *iuc*3 is associated with porcine and human clinical isolates. Recent reports of 'convergent' plasmids that carry both virulence and antimicrobial resistance genes (ARGs) have raised serious public health concern.

We fully sequenced *iuc*3-carrying IncFIB(K)/IncFII plasmids harboured by 80 Kp isolates and one *K. oxytoca* isolate recovered as part of two large 'One-Health' studies in Italy (SpARK) and Thailand (OH-DART). Adding public data gave a combined dataset representing 517 *iuc*3 isolates.

We identified six convergent plasmids from fresh markets in the Thai data, and one from a neighbouring hospital. These have emerged through the hybridisation of co-circulating *iuc*3 plasmids and plasmids encoding extended-spectrum beta-lactamases (ESBLs).

Three *iuc*3 plasmid groups were resolved, one of which is circulating in hospitals throughout Asia, with occasional examples in Europe and elsewhere. This group carries multiple antimicrobial resistance genes (ARGs), and other loci potentially involved in virulence.

Plasmid hybridisation, leading to the convergence of resistance and virulence traits, occurs frequently even in One-Health settings. Population-scale sequencing of plasmids can reveal subvariants that pose a risk to public health.

Epistasis analysis across bacterial pangenomes using Continuous-Time Markov Models

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Abstract

Nucleotide covariation in genomes can be indicative of epistasis where the function of one gene is related to that of another. Understanding these genetic interactions can provide detailed information about the mechanisms underlying important phenotypes, such as antimicrobial resistance (AMR). The power of digital microbiology for functional inference has spawned new approaches to analysing genome co-adaptation including Genome-Wide Epistasis Studies (GWES). Here we present a pangenome k-mer based approach that incorporates a bi-directional evolutionary model that weights nucleotide covariation based upon expectation according to phylogenetic relatedness of the isolates. This limits inherent sampling biases in genomic data. Applying the Continuous-Time Markov Chain (CTMC) model to construct k-mer covariation maps in pangenomes, we infer epistasis in important pathogens, including methicillin resistant Staphylococcus aureus (MRSA). Deeper CTMC analysis of MRSA evolutionary dynamics revealed the landscape of co-adaptation including lineage specific microevolution and cryptic polymorphisms linked to AMR. Developing function maps based upon realistic evolutionary models brings bioinformatics closer to traditional laboratory microbiology and has potential to inform novel approaches to understanding AMR

The Biofilm Master Regulator VpsR Modulates Virulence in *Vibrio cholerae*

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Abstract

The El Tor biotype of *Vibrio cholerae* has outcompeted the classical biotype to dominate the ongoing 64-year cholera pandemic. The key factors that have contributed to this dominance are the superior environmental persistence and the prolonged colonization of the human small intestine displayed by the El Tor biotype. Transcriptomic data indicate that El Tor has elevated cyclic-di-GMP levels under virulence conditions. In *V. cholerae*, cyclic-di-GMP signals are transduced by the transcription regulator VpsR to control gene expression. Using ChIP-seq, we mapped VpsR binding to 54 loci, with notable binding observed upstream of genes involved in biofilm formation, motility, and transcriptional regulators that control the virulence cascade. Of these, 16 genes are differentially expressed under virulence conditions compared to the classical biotype. This suggests a VpsR-dependent role in regulating gene expression during the virulence lifestyle in the El Tor biotype.

Further, we found that the VpsR DNA-binding consensus sequence shares high sequence similarity with three other master regulators of lifestyle change: CRP, HapR, and FNR. In total, 28 potential overlapping DNA-binding sites were identified, one of which is located within the vpsR promoter. At this site, cooperative binding between VpsR and CRP downregulates transcription. This example demonstrates how environmental signals can be integrated into the gene expression of a master regulator, tightly controlling the expression of its regulon. Given the conserved nature of transcription regulators across bacteria, we propose that shared DNA binding sites may represent a universal strategy for integrating regulatory signals.

Analysis of gut microbiota dynamics within families with strain-resolved metagenomics

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Abstract

Human gut microbiota is seeded at birth and most of the early colonizing species are transmitted from mothers. Previous studies have shown that kinship and cohabitation are associated with high strain transmission rates, but colonization and persistence among multiple family generations remains largely unexplored.

We investigated factors crucial for bacterial colonization and analysed strain transmission patterns between family members at an unprecedented scale. We established a meta-analysis of metagenomes from families and mother-child pairs from 17 published studies, supplemented by a local cohort. These >5,000 shotgun metagenomes were processed with the MG-TK pipeline to reconstruct metagenome-assembled genomes (MAGs), predict genes, and perform functional annotations.

We identified over 1,900 metagenomic species (MGS) clustered from >45,000 high-quality MAGs. More than 90% of these MGS were resolved intra-specifically to track strain transmissions within families and investigate their persistence over time based on longitudinal studies. This showed the strong influence family members have on each other's gut microbiome and identified species with the highest/lowest strain-level family association. Moreover, we found that despite lower diversity, strains found in newborns tend to be more persistent than those found in adults. We further analysed de novo reconstructed pangenomes for each MGS to identify genes overrepresented in newborns that could indicate their involvement in the colonization process.

Our strain-resolved analysis of metagenomes provides novel insights into strain dynamics within families and identifies genes characteristic of persistent and highly transmissible strains, indicating factors that might contribute to successful colonization.

Bacterial and plasmid interaction networks jointly shape community stability and evolution

Kaitlin A Schaal ORCID iD[1], Ying-Jie Wang ORCID iD[2], Johannes Nauta ORCID iD[3], Manlio de Domenico ORCID iD[3], Shai Pilosof ORCID iD[2], James PJ Hall ORCID iD[1]

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Abstract

There is a pressing need to understand the role of plasmids in shaping bacterial community dynamics. While studying individual plasmids and their fitness costs has taught us much, complexity theory argues that a full understanding of these processes cannot come from studying individual aspects in isolation. Our research therefore integrates interspecies interactions and plasmid interactions in a model soil community, using both modeling and experimental approaches. Our model predicts that plasmid host range can drive community composition; bacterial species that can host multiple plasmids (bridge species) are more likely to go extinct, even if the plasmids remain in the community hosted by other species. We tested this with a brief evolution experiment of a 3-species, 2-plasmid community, where each plasmid could be hosted by two species and one species could host both plasmids. We explored community dynamics across environments containing stressors relevant to resistance genes on the plasmids (kanamycin and mercury). We found that while the bridge species was unlikely to retain both plasmids simultaneously, it often persisted in the community. One species survived under MIC concentrations of antibiotic even in the absence of plasmids. Surprisingly, when this species carried a plasmid allowing it to survive the antibiotic, it was able to persist in the environment containing both stressors, even when the bridge species (which had access to both plasmids) went extinct. Overall, inter-species competition was often a more important driver in community composition than plasmid dynamics, although plasmid fitness cost did appear to contribute to species frequencies.

Pangenomic context drives mutually exclusive routes to multidrug resistance.

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Abstract

Background:

Prokaryotic pangenome variation is not random but shows highly structured associations in gene content likely driven by natural selection. How these pangenomic selective patterns relate to an organism's traits and phenotypes is still unclear, but it follows that the essentiality of any gene to a particular phenotype may also be subject to the wider genetic environment. Supporting this, we previously showed that predicting antimicrobial resistance (AMR) phenotype requires whole-genome context rather than individual AMR genes alone.

Methods:

We aimed to further understand the routes to resistance within and between species by combining pangenomics and machine learning, using *Pseudomonas aeruginosa* and *Escherichia coli* due to their clinical importance. The machine learning models revealed genes statistically linked to particular AMR phenotypes. We then searched for pairs of these key genes in networks evaluating gene presence within the pangenome (if genes are associated/disassociated).

Results:

We found that AMR genes were present within the core genome in both species. These genes often conferred resistance to multiple drug classes, suggesting multidrug resistance genes are core. We identified genes linked to multidrug phenotypes disassociated in *E. coli*, suggesting mutually exclusive routes to MDR phenotypes. We then compared *P. aeruginosa*'s pangenome network to *E. coli*'s, in which we identified that the gene pairs had different associations in the other networks. This highlights the importance of understanding the genetic context of AMR mechanisms to explore how AMR arises in different strains/species, which will be a key component to tackling the increase of AMR.

Mechanisms underpinning evolution and adaptations to inhibition of cell cycle regulator, Cdc25 phosphatase, in the yeast model organism *Schizosaccharomyces pombe.*

Chantelle Endeley ORCID iD[1,2], Maria Domingo-Sananes ORCID iD[1]

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Abstract

The cell cycle consists of distinct, sequential phases which enable the orderly division of a cell into two identical daughter cells. Entry into the mitosis phase is governed by the activation of Cdc2 by Cdc25 phosphatase. Overexpression of Cdc25 in cancers leads to aberrant cell proliferation and is associated with poor prognoses in patients. As such, therapeutic inhibition of Cdc25 is being investigated as a strategy to treat cancer. However, as cancers can evolve and adapt in response to treatment, identifying resistance mechanisms is pertinent to uncover targets that may limit disease progression and patient relapse.

Here, we use the fission yeast *Schizosaccharomyces pombe* as a model organism to investigate mechanisms of adaptation when Cdc25 function is impaired. We performed experimental evolution across 200+ generations on two mutant *S. pombe* strains with limited Cdc25 function – *cdc25-ts* (temperature sensitive) and *cdc25-13A* (lacks activation by Cdc2). The evolved populations were characterised to assess changes in the phenotype and genotype compared to ancestor strains.

We observed that the fission yeast strains adapted to decreased Cdc25 function. Cell size and generation time decreased in the evolved populations of both mutants, and a loss of temperature sensitivity was observed in the evolved *cdc25-ts* populations. Whole-genome sequencing showcased distinct differences in mechanistic pathways of adaptation. The *cdc25-ts* populations acquired mutations in the cell cycle regulators, *cdc2* and *wee1*, while the *cdc25-13A* populations mostly acquired mutations in *mts4*, a regulatory subunit of the 26S proteosome. Work continues to validate these as mechanisms of adaptation.

Inter- and intra-patient evolution of a cystic fibrosis epidemic strain of *Pseudomonas aeruginosa* over the last 40 years.

Fiona Whelan ORCID iD[1], Barb Waddell[2], Michael Parkins[2], Michael Surette[3]

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Abstract

Infection by epidemic transmissible strains of *Pseudomonas aeruginosa* are associated with worse clinical outcomes in people with cystic fibrosis (pwCF). The Prairie Epidemic Strain (PES) of *P. aeruginosa* was recently described in the prairie provinces of Canada. Using an established biobank of *P. aeruginosa* strains collected from pwCF, we have subsequently sequenced every PES strain in the collection: 440 genomes from 67 pwCF. The oldest isolate of PES dates to 1980 yet phylogenetic evidence suggests that this clone was circulating in the population from an earlier date. We identify a remarkably stable pangenome with very little gene gain or loss and some recombination over >40 years of inter- and intra-pwCF evolution. Across all PES genomes, we identified particular regions of the genome with increased SNP density; this includes particular genes (e.g., pvdS, mexT, lasR, mucA) recently linked with host adaptation and others (e.g., PA1874) associated with transmissibility. We identify the emergence of hypermutators in a subset of PES strains restricted to a small number of pwCF and evidence of intra-pwCF selective pressures. A better understanding of how transmissible strains of *P. aeruginosa* evolve will help us mitigate their spread and effect on pwCF.

Industry-sponsored sessions

High-Throughput Single-Microbe Genomics Enabled by Semi-Permeable Capsules

Vaida Kurmauskaite, Gabija Lauciute, Domas Rupkus, Simonas Jocys, Zana Kapustina, Rapolas Zilionis

Atrandi Biosciences, Vilnius, Lithuania

Abstract

Single-cell DNA sequencing complements the metagenomic analysis of uncultured bacteria by revealing cell-to-cell variation, linking host genomes to extrachromosomal DNA, and providing strain-level taxonomic resolution. However, current techniques are limited to processing fewer than 1,000 cells and produce single-amplified genomes (SAGs) of low completeness due to biases in whole genome amplification (WGA).

We present an innovative, cost-effective approach to sequence up to 10,000 SAGs with superior genome recovery. Individual microbial cells are isolated into 70 µm semi-permeable capsules (SPCs), enabling compartmentalized multi-step processing - including lysis, WGA, and barcoding - of all cells simultaneously at a cost of less than $1 per cell. Using well-characterized E. coli and B. subtilis, we demonstrate >90% genome recovery per SAG at sequencing depths below 10x and <1% cross-contamination. Additionally, we processed a commercially available microbial community standard and were able to detect all species within the mixture.

The advantages of SAG sequencing are especially valuable for uncovering the diversity and unique adaptations in the context of microbial ecology.
We processed soil and aquatic samples to generate matched SAG and MAG datasets, with the latter obt ained through bulk metagenomics.
SAG assemblies produced longer contigs and revealed detailed genomic features, most notably the linkage between viral and plasmid sequences and their
hosts. The linkage information was lost in the MAG dataset.

Our high-throughput SAG sequencing workflow provides a detailed view of microbial communities, offering unmatched resolution and scalability. This capsule-based single-cell sequencing technology opens new horizons for microbial genomics research.

Enhance Microbial World Exploration with Standardised Sample Prep

Dr. Véronique Karsten

MP Biomedicals, Illkirch, France

Abstract

Dr. Véronique Karsten, product manager of the Molecular Biology product portfolio at MP Biomedicals, will explain how to thoroughly homogenize and lyse microorganisms in samples for microbiome research. She will also discuss new DNA and RNA extraction kits from MP Biomedicals for high yields from diverse samples, including environmental, water, plant, fecal, and tissue specimens.

Key learning objectives

- Learn how to obtain a thorough homogenization and lysis of any microorganism from samples used in microbiome research
- Explore the new DNA and RNA extraction as well as coextractions kits developed by MP Biomedicals to generate high yields and achieve rapid isolation of genomic DNA and total RNA from environmental and water samples, plant specimens, feces, human and animal tissues
- Discover MPure-32™ and MPure-96™ Automatic Nucleic Acid Purification Platforms designed for high-throughput applications and combined kits specially developed for DNA and RNA purification from various samples including feces and environmental samples

Advancing microbial genomics with highly accurate long-read sequencing

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Abstract

Long-read sequencing technologies have revolutionized microbial genomics by providing greater depth, resolution, and completeness of genomic data. Among these, PacBio HiFi sequencing stands out as a single, comprehensive technology that offers highly accurate long reads while preserving critical epigenetic information, such as DNA methylation patterns. This high accuracy, combined with long-read capabilities, makes it a powerful and cost-effective tool for microbial genomics that enables researchers to generate high-confidence genome assemblies that were previously challenging with short-read technologies.

Its single-technology approach ensures high accuracy, data completeness, and methylation detection, enabling high-quality whole-genome sequencing (WGS) of microbial isolates. HiFi sequencing delivers reference-grade assemblies with closed chromosomes and plasmids, reducing the need for additional scaffolding or polishing.

For microbiome profiling, HiFi full-length amplicon sequencing (e.g., 16S, ITS, 18S) offers superior taxonomic resolution at the species and strain level by sequencing entire genes. This improves classification accuracy and enables precise microbial identification.

Additionally, HiFi shotgun metagenomic sequencing enhances functional insights and taxonomic profiling, reducing misclassification errors while enabling a deeper understanding of metagenomes. HiFi reads also improve metagenome-assembled genome (MAG) recovery, generating more complete and single-contig MAGs, even at lower coverage. This is particularly valuable for studying hard-to-culture or low-abundance microbes.

By providing high accuracy, completeness, and epigenetic insights, PacBio HiFi sequencing advances microbial genomics research, from isolate genome assembly to complex metagenomes. Its ability to generate high-quality, contiguous sequences with epigenetic information makes it a cost-effective and scalable solution for researchers studying microbial diversity, evolution, and function in a variety of environments.

Infection Forum

Infection Science Awardee: The spatio-temporal localisation of a pan-Mucorales specific antigen: a promising immunohistochemistry target and potential biomarker for mucormycosis

Alyssa Hudson[1,2], Dora Corzo-León[1], Iana Kalinina[1], Duncan Wilson[1], Christopher Thornton[3,4], Adilia Warris[1], Elizabeth Ballou[1]

[1]Medical Research Council Centre for Medical Mycology at the University of Exeter, Exeter, United Kingdom. [2]Royal Devon University Hospitals NHS Foundation Trust, Exeter, United Kingdom. [3]Biosciences, Faculty of Health and Life Sciences, University of Exeter, Exeter, United Kingdom. [4]ISCA Diagnostics Ltd., Hatherly Laboratories,, Exeter, United Kingdom

Abstract

Background: Mucormycosis is an aggressive, invasive fungal infection caused by moulds in the order Mucorales. Early diagnosis is key to improving patient prognosis, yet currently relies on insensitive culture or non-specific histopathology. A pan-Mucorales specific monoclonal antibody (mAb), TG11, was recently developed (Thornton *et al.* 2023).

Methods: Our study characterises the spatio-temporal localisation of the antigen recognised by TG11 using immunofluorescence (IF) microscopy and time-lapse imaging of *Rhizopus arrhizus*, and localisation in a further ten Mucorales species of clinical importance. Immunogold transmission electron microscopy (immunoTEM) reveals the sub-cellular location of mAb TG11 binding. Finally, we used mAb TG11 to perform IF microscopy of *R. arrhizus* and *Aspergillus fumigatus* in an *ex vivo* murine lung infection model.

Results: IF of fixed cells revealed TG11 antigen production at the emerging hyphal tip and along the length of growing hyphae in all Mucorales except *Sakasenea*. The TG11 mAb did not bind to ungerminated spores except *Syncephalastrum*. Timelapse imaging revealed early antigen exposure during spore germination and along the growing hypha. ImmunoTEM confirmed mAb TG11 binding to the hyphal cell wall only. The TG11 mAb specifically stained *R. arrhizus* hyphae in infected murine lung tissue, and did not detect *A. fumigatus* hyphae, demonstrating utility as a specific immunohistochemical stain for invasive Mucorales hyphae.

Conclusions: TG11 detects early hyphal growth and has valuable potential for diagnosing early Mucorales infection and differentiating infection from colonisation or sample contamination. TG11 enables specific histopathological detection of Mucorales in tissue and differentiates Mucorales from the main differential diagnosis, *Aspergillus*.

Infection Science Awardee: Last line treatment options for Carbapenemase-producing Enterobacterales: a call for harmonization of cefiderocol breakpoints

Ioannis Baltas ORCID iD[1,2], Trupti Patel[1], Ana Lima Soares[1]

[1]University College London Hospitals NHS Foundation Trust, London, United Kingdom. [2]UCL Institute of Child Health, London, United Kingdom

Abstract

Introduction

Carbapenemase-producing Enterobacterales (CPE) pose difficult therapeutic challenges. We aimed to characterise antimicrobial resistance profiles of CPE in our centre.

Methods

All consecutive non-duplicate CPE isolates between 01/08/2020 and 31/08/2023 were retrospectively studied. Cefiderocol antimicrobial susceptibility testing (AST) was performed using disc diffusion, ceftazidime-avibactam using disc diffusion and gradient diffusion, ceftazidime-avibactam aztreonam synergy using the double disc diffusion method. EUCAST version 14.0 breakpoints were used and compared to 2023 FDA/CLSI breakpoints.

Results

A total of 158 CPE from 136 patients met the inclusion criteria. OXA-48 was the most prevalent carbapenemase (48.1%), followed by NDM (38%). 69.7% of NDM-producing isolates were resistant to cefiderocol, while a further 18.2% in the Area of Technical Uncertainty (ATU). Ceftazidime-avibactam and aztreonam synergy was seen in 87.5% of isolates, while colistin and fosfomycin susceptibility remained high (98.1% and 97.2% respectively). All OXA-48-producing isolates were susceptible to ceftazidime-avibactam, and 15.3% were resistant to cefiderocol. If the 2023 FDA/CLSI disc diffusion breakpoints were applied only 2.7% (3/113) of all isolates would have tested resistant to cefiderocol, (4.5%, 3/66 of NDM-producers and none of the OXA-48 producers).

Conclusion

We found high rates of resistance to cefiderocol in CPE isolates without prior cefiderocol exposure using EUCAST version 14.0 breakpoints. Harmonisation of EUCAST and FDA/CLSI cefiderocol breakpoints is urgently needed as current differences are likely to lead to significant variation in the use of cefiderocol in countries adopting breakpoints from one or the other regulator.

Infection Science Awardee: Pre-analytical sampling and processing of tongue swabs for Mycobacterium tuberculosis complex (Mtbc) impacts diagnostic yield

Helen Savage[1,2], Chifundo Salifu[3], Mavis Menyere[3], Thomas Edwards[1], Alice Mnyanga[4], Catherine Anscombe[3], Elizabeth Corbett[5], Naomi Walker[1], Peter MacPherson[6]

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Abstract

Background: Oral swabs are an alternative diagnostic sample to detect Mycobacterium tuberculosis complex (Mtbc). Previous studies have processed samples remotely in overseas laboratories. We aimed to optimise pre-analytical sampling methods at point of collection in Blantyre, Malawi. We compared self- and healthworker-taken tongue swabs, analysed using Xpert Ultra and RT-PCR, and compared by cryopreservation method.

Method: Participants (adults with newly diagnosed sputum Xpert confirmed pulmonary TB) were randomly allocated to undergo self- or healthworker swab first. Two swabs were taken (COPAN FLOQswab in Primestore MTM media 1.5 ml) then tested for Mtbc by Xpert Ultra after storage at either room temperature or -80 C, and by RT-PCR after one or two freeze-thaw cycles.

Results: In total 100 tongue swabs were taken (50 self-taken, 50 healthworker-taken) with an overall sensitivity of 36% on Xpert Ultra and 43% on RT-PCR compared to Xpert in sputum. Samples that underwent two freeze-thaw cycles prior to RT-PCR had a significantly higher sensitivity 17/28 (60.7%) compared to one cycle 26/72 (36.1%) on RT-PCR (p=0.04). There was no difference in sensitivity between self-taken and healthworker-taken swabs.

Conclusion: When analysing tongue swabs for Mtbc additional freeze-thaw cycles may cause increased lysis leading to mycobacterial DNA release and an increase in sensitivity on RT-PCR. When swabs are transported for analysis incorporating freeze-thaw cycles, as opposed to processing at point of collection, this may increase sensitivity. This needs to be accounted for as additional feasible and affordable lysis steps may need to be added to protocols at point of collection.

A natural product cocktail derived from an “ancientbiotic” with enhanced activity against Staphylococcus aureus and Pseudomonas aeruginosa biofilms

Oluwatosin Orababa ORCID iD[1], Jessica Furner-Pardoe[1], Alexander Gale[1,2], Christophe Corre[1], Freya Harrison[1]

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Abstract

Biofilm infections like chronic wounds and cystic fibrosis (CF) lung infections are of great concern because they are highly resistant to antibiotics and require high antibiotic concentrations to clear them. We aim to develop a natural product cocktail from a complex medieval infection remedy—"Bald's eyesalve"—with enhanced antibiofilm efficacy against bacterial biofilms, and to explore its mechanism of action. The original remedy is a combination of four chemically-complex natural ingredients whose optimum antibiofilm activity is seen only when all ingredients are present. Based on the original formulation, we developed a simpler version of this complex remedy with a more defined chemical composition and optimised activity against bacterial biofilms. We report that this newly formulated cocktail has enhanced activity against biofilm-associated populations of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in host-mimicking models compared with the original remedy. This cocktail caused more than 4-$\log_{10}$ killing of *S. aureus* (methicillin-susceptible and resistant strain) and *P. aeruginosa* in an *in vitro* soft tissue biofilm model. Additionally, we observed a complete eradication of *P. aeruginosa* biofilms in an *ex vivo* cystic fibrosis lung model. We also report that Bald’s eyesalve-derived cocktail causes significant disruption to bacterial membrane potential and irreversible damage to the bacterial cell membrane. Lastly, both *S. aureus* and *P. aeruginosa* showed reduced resistance evolution to this new cocktail compared to mainline antibiotics. We have shown that this natural product cocktail could potentially be developed into a good treatment for chronic bacterial biofilm infections, including chronic wounds and CF lung infections.

Unveiling the Complexities of Microbial Interactions in the Cystic Fibrosis Airways in Response to Therapeutic Interventions

Pok-Man Ho ORCID iD[1], Rahan Rudland Nazeer[1], Robert A Quinn ORCID iD[2], Martin Welch ORCID iD[1]

[1]Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom. [2]Department of Biochemistry and Molecular Biology, Michigan State University, Michigan, USA

Abstract

In treating infections, we rely on antimicrobial drugs to eliminate pathogens. However, patients with chronic infections, such as people with Cystic Fibrosis (pwCF), often experience suboptimal responses to these therapies. This challenge is compounded by the polymicrobial nature of their infections and the use of multi-drug regimens, which complicates treatment compared to monospecies infections. The rise of antimicrobial resistance further underscores the need to understand how each medication affects the airway microbiota. Furthermore, microbes may exhibit resistance to certain antimicrobials in polymicrobial contexts, despite being sensitive when cultured in isolation, leading to a disconnect between expected and actual drug efficacy and increasing the risk of genetic resistance.

To understand this, I developed a computational model to capture key elements of microbial ecology, based on the generalized Lotka-Volterra model enhanced by a Bayesian Inference adaptive Markov Chain Monte Carlo framework. By analysing UK-wide time-series data from the CF registry, I have identified unique ecological signatures linked to various therapeutic interventions. My machine learning approach reveals dynamic interactions both within- and across time-windows. Recent findings from metagenomic time-series data confirm that different CF medications exert distinct ecological effects on the microbial interactome, and that these may vary among individuals. This systematic method allows for the quantification of interactions between microbial species and the specific impacts of multi-drug therapies. Ultimately, my research offers a novel perspective on the influence of antimicrobials on complex microbial communities, paving the way for personalised therapeutic strategies.

Cross-scale infection imaging: visualising infection and pathobiology from single cells to whole organs

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Abstract

Visualising infection dynamics across spatial scales is key to understanding the interplay between pathogens, the host, and the efficacy of clinical interventions. The difficulty in capturing the infection dynamics on a global scale lies in the lack of accessible imaging technologies to assess large tissue specimens with the high spatial resolution required to resolve individual pathogens. We present three separate case studies, where we have developed and applied innovative specimen preparation, imaging, and analysis methods to study infection biology across multiple spatial scales; (1) a high-efficiency optical clearing method for imaging whole *Galleria mellonella* larvae for the study of host-pathogen interactions, (2) quantitative multi-scale imaging to understand how anti-virulence therapies against enteric pathogens confer a protective role in renal remodelling, and (3) unprecedented visualisations of entire wound beds infected by polymicrobial biofilms and quantifying the spatial dynamics of wound infections in three dimensions. The three cases we present exemplify how cross-scale and multi-modal microscopy approaches offer new insights into colonisation, infection, and intervention studies. We propose that the open methods we have developed have great potential for translation to other infection biology scenarios, thereby opening the doors for microbiologists and immunologists to address key questions that span multiple length scales.

Rapid genetic adaptation to harsh acidic conditions by a clinical isolate of Listeria monocytogenes reveals selection on non-canonical start codons for elevated SigB activity.

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Abstract

Listeria monocytogenes is a major food-borne pathogen that causes life-threatening disease. SigB mediates its host entry by promoting acid resistance and epithelial attachment. Surprisingly, a clinical isolate MQ140025 (from hypervirulent clone) manifested two mutations affecting the SigB activation resulting in reduced SigB activity. We hypothesized that the plasticity in SigB activation cascade can contribute to conditional fitness advantage. To test this, MQ140025 was subjected to four acid-challenge and overnight-recovery cycles. Acid resistance rapidly developed in all three tested cultures and two acid resistant derivatives (ARD) were isolated from each culture. SigB activities were restored in all ARDs due to mutations acquired in *rsbW*, which encodes an SigB antagonist. These mutations resulted in non-canonical start codons ($rsbW^{ATG}$ to $rsbW^{ATA}$/$rsbW^{ATT}$) or premature translation termination ($rsbW^{-}$). Interestingly, ARDs carrying $rsbW^{ATT}$ or $rsbW^{-}$ were SigB hyperactive and appeared as small colony variants. Translational reporter assay demonstrated distinct differences in translation efficiency among three start codons: ATG>ATA>ATT, suggesting that perturbations of RsbW:SigB stoichiometry can alter SigB activity. The start codon usage was then systematically examined for 60,692 *L. monocytogenes* genomes from NCBI database. This analysis revealed that non-canonical start codons can be selected for virulence- and stress-related genes and, in some cases, associated with hyper- or hypo-virulent genetic clades. Taken together, we show the genetic plasticity of stress response regulation in a *L. monocytogenes* strain of clinical relevance, and highlight the importance of translational control as means of fine-tuning gene expression during short-term adaptation and long-term evolution for optimal fitness.

Adaptation of *Campylobacter* to Sodium Chloride Alters Phenotypes Associated with Human Disease

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Abstract

Campylobacter spp. live as commensals in chicken intestines but are the leading cause of human bacterial foodborne gastroenteritis worldwide. Consumers are exposed to *Campylobacter* via undercooked or raw poultry meat. Pre-marinated chicken products that contain a variety of additional seasonings, like salt, are becoming increasingly popular. Limited studies have been conducted on the effects of long-term exposure to salt on *Campylobacter.* The aim of this study was to expose a panel of *Campylobacter* isolates to sodium chloride and assess if adaptation to salt altered any phenotype that would increase the risk of human infection. Five strains were adapted by growing on solid and in liquid media supplemented with 1.25% (w/vol) and 1.5% (w/vol) sodium chloride respectively. Motility and catalase activity were similar between wild type and salt adapted strains. Biofilm formation as assessed by crystal violet staining and fluorescent microscopy revealed that adaptation to salt resulted in a reduction of biofilm mass but an increase in biofilm viability. Autoagglutination and lipooligosaccharide (LOS) profiles, phenotypes associated with adhesion and invasion, were altered between wild type and salt adapted strains. DNA supercoiling, which has been associated with the regulation of virulence in *C. jejuni,* was altered by growth in broth supplemented with 1.5% sodium chloride. In conclusion, adaptation to salt results in changes to phenotypes associated with virulence in humans such as biofilm formation, autoagglutination, and alteration in LOS profiles. DNA supercoiling may play a role in modification of the phenotype of *Campylobacter* after exposure to salt.

The V-ATPase as a sensor of pathogen-associated pH neutralisation

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Abstract

Multiple pathogens cause the dissipation of pH gradients within host intracellular compartments. Examples include bacteria expressing pore-forming toxins, such as listeriolysin O in *Listeria monocytogenes*, and viroporins such as influenza M2 and SARS-CoV-2 E protein. As a common feature across many infections, pH neutralisation represents a candidate pathogen-associated molecular pattern that could warn host cells of the presence of an infectious agent.

Recent work in our group has shown that host cells sense compartment deacidification via the vacuolar ATPase (V-ATPase), which recruits the autophagy adaptor ATG16L1 to induce decoration of the damaged compartment with ubiquitin-like ATG8 proteins. Indicating an important role in infection, this process is specifically blocked by the *Salmonella* effector SopF, which ADP-ribosylates the V-ATPase to prevent ATG8 lipidation. Infection-associated host proteins such as the interferon-stimulated gene NCOA7 have also been shown to bind to the V-ATPase, indicating a potential role in modulating this process.

We aim to investigate the V-ATPase as a signalling hub in infection, using model pathogens that are known to disrupt intracellular pH. Using the *Legionella pneumophila* effector SidK, which binds to the V-ATPase subunit ATP6V1A with very high affinity, we will immunoprecipitate V-ATPase complexes from cells infected with influenza virus. We will then use liquid chromatography and tandem mass spectrometry to analyse the proteins that co-precipitate with the V-ATPase. We hope to identify novel interactors involved in sensing compartment deacidification, which could play a role in the host response to a wide range of intracellular pathogens.

Spatially resolved host-pathogen interactions in chronic lung disease

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Abstract

In chronic lung diseases (CLD) such as cystic fibrosis (CF), impaired clearance systems and mucus accumulation favour local microbial replication and high microbial load, which drives a destructive cycle of lung damage. Within-host evolution studies have shown evidence of parallel evolution in key virulence systems in classical CF pathogens *P. aeruginosa* and*M. abscessus.* The potential impact of these host-adapted clones on lung cells is poorly understood since a vast majority these studies have used sputum and thus lack a complete view of distribution of microbial diversity across the lung. To gain spatial resolution of bacterial profiles and cellular niches across the lung, we sampled 8 anatomically distinct lung sections from a single patient with CF with chronic infection. Tissue histopathology revealed numerous granulomas and mucus filled airways. Pathogen WGS showed clustering of pre and post transplantation *M. abscessus* isolates and non-synonymous mutations in key virulence systems (*mmpL*, *pstA, lgrD)*. Taxonomic profiling by bulk RNAseq identified key CF pathogens *Pseudomonas spp*., *Mycobacterium spp*., *Staphylococcus spp*., and interestingly, *Klebsiella spp*. We noted significant variation in bacterial profiles across the lung. We designed a custom probe panel against 27 genes of *M. abscessus* and *P. aeruginosa* and using spatial transcriptomics we co-capture pathogen and host transcriptomes. Our analyses revealed a three-layer granuloma structure and heterogeneity of *M. abscessus* granulomas within a patient. This work provides a foundation for the identification mechanisms through which pathogens drive lung damage, thus potentially identifying novel therapeutic targets and improving clinical interventions.

Proteomic dissection of Aspergillus fumigatus – host interactions using the ex-vivo pig lung model

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Abstract

Aspergillus fumigatus is an environmental saprophyte and an opportunistic pathogen of the human airway. A. fumigatus can cause chronic infections, typically in the context of pre-existing lung damage. Animal models of infection are costly and often cannot recapitulate phenotypes observed in patients. The ex-vivo pig lung (EVPL) model was developed for conducting bacterial infection studies as pigs share 90% immunological homology to humans and display many anatomical similarities. EVPL also retains resident immune cells and richer cellular complexity compared to in-vitro models, in addition to a microbiome. Proteomic analysis of infected and uninfected tissue enabled the tracking of molecular changes to the pathogen and the host during the establishment of fungal infection. Analysis identified the metabolism and development of Aspergillus on the EVPL sections mimicked patterned observed in clinical isolates shifting from initial carbon metabolism to protein metabolism with an emphasis on amino acid metabolism and biosynthesis, which in turn fuel the production of fungal virulence factors such as mycotoxins. The tissue remained responsive to the pathogen with proteins increased in abundance associated with innate immune recruitment at 24 hours, while proteins associated with neutrophil degranulation were decreased in abundance. At 96 hours the infected tissue demonstrated enhanced expression of fibrotic markers relative to the uninfected control. These similarities in response validates the use of this model and contributes supporting evidence that these patterns are of clinical importance during A. fumigatus colonisation and infection of pulmonary tissue.

The interaction of L. monocytogenes with human placental macrophages changes as gestation proceeds.

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Abstract

Hofbauer cells (HBC) are extra-embryonic macrophages generated *de novo* within the placenta. As they are the only nascent immune cells normally found in the human placenta, it is reasonable to suppose that they function to protect the fetus from vertically-transmitted pathogens. Here, we sought to determine how the properties of HBC change throughout gestation in the absence of known pathogens, and in response to infection with to *Listeria monocytogenes*, a pathogen that crosses the placenta and replicates in macrophages. We find there are substantial transcriptomic changes between first trimester and term HBC, and many of these genes are involved in immune responses. As gestation progresses, we find that the HBCs decrease the maturation and acidification of their phagosomes. The observed decrease in HBC phagosome maturation and acidification is accompanied by reduced colony forming units and actin recruitment by *Listeria monocytogenes* in first trimester HBC in comparison to term. These findings are consistent with an impaired capacity for *Listeria monocytogenes* to escape from vacuoles in first trimester HBC. Overall, this study demonstrates that the differences between first trimester and term HBC impact on their interactions with *Listeria monocytogenes*.

Toxic Trickery: Antitoxin-induced auto-phosphorylation blocks bacterial toxin activity

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Abstract

Mycobacterium tuberculosis encodes the largest number of toxin-antitoxin (TA) systems of any known bacterial species, suggesting a causative link between TA systems and success as an infectious agent. The MenAT family of TA systems represent four such systems, encoding MenT nucleotidyltransferase (NTase) toxins and diverse MenA antitoxins. MenT toxins transfer nucleotides to the 3'-CCA motif of specific tRNA targets, inhibiting aminoacyl charging and blocking cellular translation, thereby arresting bacterial growth. RNA sequencing and autoradiography revealed the nucleotide preference and tRNA specificity of related MenT toxins, elucidating key structural features governing specificity of NTase activity. MenA antitoxins can bind to and sequester MenT toxins, forming stable toxin-antitoxin complexes in solution. However, antitoxin binding also induces a conformational change to the catalytic site of the toxin, triggering toxin auto-phosphorylation. Crystallographic studies revealed phosphorylation of strictly conserved toxin residues results in reduced electropositive charge and steric occlusion within the nucleotide-binding pocket, the result of which is an inhibition of NTase activity, thus restoring bacterial growth. The ubiquity of related NTases amongst prokaryotes raises the possibility that this novel mode of antitoxicity represents a widespread neutralisation mechanism responsible for governing the activity of toxins used by human pathogens.

Dissecting the crosstalk between stress granules and yellow fever virus infection

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Abstract

The phase separation of biomolecules into so-called stress granules (SGs) allows the cell to tightly regulate translation activity in response to different stimuli, such as the recognition of viral RNAs. In this context, the formation of SGs has been observed during infection with various viruses. However, recent reports suggest that in addition to their role in translational regulation, SGs modulate additional cellular processes such as the innate immune response. Here, we aim to dissect the role of SGs in the context of yellow fever virus (YFV) infection. We find that, in contrast to other flaviviruses such as ZIKV, YFV does not induce translational shut-off. Further comparison of the egg-adapted vaccine strain YFV/17D with the highly pathogenic YFV/Asibi strain shows that only infection with the highly pathogenic strain induces the formation of SGs. SG formation is presumably modulated by viral proteins, as we find that ectopic expression of the viral capsid can inhibit sodium arsenite-induced SG formation. This is further supported by biochemical interaction studies showing that the YFV capsid can bind to the major SG scaffolding protein G3BP1. Interestingly, proteomic analysis of YFV/Asibi induced SGs reveals infection-specific sequestration of pro- and anti-viral factors within these granules. In addition, many sequestered proteins appear to be metabolically linked to mitochondrial activity. In this regard we find that YFV infection indeed influences mitochondrial fragmentation and activity. Taken together, these results suggest that YFV-induced SGs modulate mitochondrial activity and possibly viral replication independently of their function in translational control.

Signalling and Warfare in a Polymicrobial Community

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Abstract

The Post-Antibiotic Era describes the rise in antimicrobial resistance (AMR) and the lack of novel antibiotic discovery. Thus, alternative methods are required for effective pathogen control. Small molecules have gained considerable traction in selective pathogen targeting and can be viewed as an anti-infective strategy. The chemical languages evolved in dominant pathogenic organisms could offer a novel strategy for suppression of key virulence phenotypes and competitiveness in co-colonising fungal and bacterial pathogens.

One major challenge to the clinical development of anti-virulence interventions is that microbes exist in diverse polymicrobial communities that undergo significant genotypic and phenotypic diversification. Co-colonising microbes present in the cystic fibrosis (CF) lung, encompassing bacterial (*Pseudomonas aeruginosa*) and fungal (*Candida albicans*, *C. dubliniensis* and *Aspergillus fumigatus*) lab and clinical strains were studied in response to host/microbial signals including *N*-acetyl-glucosamine (NAG), bile, and bile salts. Fungal and bacterial pathogens were found to alter their virulence through biofilm formation, pigmentation, and toxin production in response to specific host signals, adopting distinct morphological and pigmentation profiles when co-cultured in the presence of competing organisms. Emergence of distinct pigment-secretion and pigment-retention profiles indicated a context-dependent shift in pathogen behaviour.

Selective control of these mixed consortia and their distinctive pigmentation patterns was explored both phenotypically and via proteomic analysis using a range of small molecule frameworks. While some retained activity similar to the individual species studies, others were diminished in their anti-*P. aeruginosa* potency when other ESKAPE or fungal pathogens were present.

Knocking Out AMR Forum

Investigating the Convergence of Hypervirulence and Multi-Drug Resistance in Klebsiella pneumoniae in Sudan

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Abstract

Introduction: Multi-drug resistant (MDR) and hypervirulent (hvKp) *Klebsiella pneumoniae* have historically existed as distinct subgroups. However, observed convergence of the two phenotypes has caused significant concern. Phenotypic testing, such as the 'string test', is commonly used to detect hvKp, as an alternative for more resource-intense surveillance strategies. However, the specificity and sensitivity of the string test has been questioned. This study aimed to investigate potential hvKp and MDR *K. pneumoniae* convergence using genome analysis.

Methods: Whole-genome sequencing (WGS) data from twenty MDR *K. pneumoniae* isolates from Khartoum, were analysed using Kleborate to identify virulence genes such as *iuc*, *ybt* and *rmp*, and acquired β-lactamases such as CTX-M-15, OXA-48 and NDM-5. The sequences were analysed using PlasmidFinder to identify potential plasmids within the genomes. The string test was used to assess its suitability for detecting hvKp.

Results: All strains had the *ybt* gene, five of which harboured additional virulence genes such as *rmp* and *iuc* indicating the presence of convergent MDR/hvKp in Sudan. A hybrid MDR/hvKp plasmid (IncHI1BIncFIB), containing CTX-M-15 and NDM-5 alongside *rmp* and *iuc*, was found in one isolate. The string test was found to have no statistically significant correlation with numerous virulence factors identified in the study, including the *rmp* gene linked to hypermucoidity.

Discussion: The data shows convergent MDR and hvKp strains from Khartoum, Sudan. The identified plasmid suggests the need for enhanced genomic surveillance of convergence in clinical settings. This study emphasises the need to refine techniques for defining hypervirulent *Klebsiella pneumoniae*.

Clinical and veterinary isolates of the *Klebsiella planticola* and *terrigena* complexes contribute to the global burden of β-lactamases

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Abstract

The *K. planticola* and *K. terrigena* complexes (KplC and KtC, respectively) represent emerging clinical and veterinary pathogens. Identification of ornithine decarboxylase-negative veterinary isolates of *K. ornithinolytica* among our in-house strains led us to undertake in-depth genomic analyses of these little-studied bacteria. Outputs from a *Klebsiella* genus-wide pangenome analysis were used as the basis for *in silico* functional (COG, KEGG) analyses. A reference database of recognized PLA (6 protein sequence variants), ORN (6 variants) and TER (2 variants) b-lactamases was created informed by the literature and the Beta-Lactamase DataBase, and was also used to interrogate the pangenome data. With an extended analysis of KplC and KtC genomes (*n* = 1,005; RefSeq and AllTheBacteria), new PLA, ORN and TER classifications were determined based on non-redundant amino acid and nucleotide sequences, with a synthetic biology approach used to characterize the novel class A b-lactamases that we identified. KplC and KtC species are functionally more similar to *K. pneumoniae* than other *Klebsiella* spp. PLA (32 variants) and ORN (39 variants) are core to the KplC, with novel core b-lactamases predicted for *K. electrica* and *Klebsiella* sp002270295 (4 sequence variants each). TER was core to the KtC (36 variants). The novel class A b-lactamases we identified are functional, with the synthetic biology approach we have adopted amenable to scale-up to provide a high-throughput means of gaining greater insights into the functional capabilities of *Klebsiella* spp. and improving the accuracy of predictions made by bioinformatics tools used for resistome profiling.

Adaptive Laboratory Evolution to Induce Antibiotics Production of *Streptomyces* sp. SSUT88A against Drug-Resistant Bacteria

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Abstract

The rapid rise of multidrug resistance (MDR) poses significant challenges for the treatment of various infections, underscoring the need for the exploration of new antibiotics targeting MDR pathogens. *Streptomyces* spp. is a major promising source of novel antibiotics. In this study, the laboratory evolution adaption of *Streptomyces* sp. SSUT88A was conducted by co-cultivating with drug-resistant pathogens for three cycles of 10 days each to stimulate antimicrobial substance production. Antimicrobial activity of adapted strains and wild-type were assayed using agar-well diffusion method. The yield of crude extracts from adapted strains and wild-type were also examined. Methicillin-resistant *Staphylococcus aureus* (MRSA) showed highest potential to compete and induce the production of crude antimicrobial compound of *Streptomyces* sp. SSUT88A after third cycle of adaptation, showing 72.58% higher crude production than the wild-type. The most improved antimicrobial activities of adapted SSUT88A against MRSA were observed in adaptations of SSU88A co-cultured with *Acinetobacter baumannii* (SSUT88A^{Ab}) and SSU88A co-cultured with MRSA (SSUT88A^{MRSA}), which were greater than the wild-type strain by 97.87% and 70.21%, respectively. The adapted strains not only effectively inhibited the growth of the MRSA but also exhibited enhanced antimicrobial activity against methicillin-resistant *Staphylococcus epidermidis* (MRSE). Interestingly, the crude compounds from SSUT88A triggered by different pathogens produced a diverse antimicrobial activity profile. *Pseudomonas* sp. N90PS did not appear to be an effective selection factor for enhancing crude compound synthesis against Gram-positive pathogens (MRSA and MRSE). However, it demonstrated a minor induction of certain crude compound compositions that inhibited the growth of Gram-negative drug-resistant pathogens.

Impact of early life antibiotic and probiotic treatment on gut microbiome and resistome of preterm infants

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Abstract

Preterm infants (<37 weeks gestation) are often administered broad-spectrum antibiotics in hospitals due to their vulnerability to severe infections, including necrotising enterocolitis and sepsis. However, antibiotics can disrupt the development of early-life microbiota, potentially impairing gut immunity and colonisation resistance. Evidence shows that probiotics (e.g., *Bifidobacterium*) may help restore healthy gut microbiota. In this study, we examined the effects of probiotics and antibiotics on the preterm gut microbiome and resistome in two unique cohorts of 34 very-low-birth-weight, human-milk-fed preterm infants, with one cohort receiving probiotics. Within each group, some infants were treated with antibiotics (benzylpenicillin and/or gentamicin) while others served as controls. We performed shotgun metagenome sequencing on 93 longitudinal faecal samples, generated >300 metagenome-assembled genomes, and obtained ~90 isolate genomes through targeted culturomics, enabling analysis of the microbiome/resistome at species and strain levels. Additionally, we investigated *in vitro* horizontal gene transfer (HGT) capacity of the multidrug-resistant (MDR) pathogen *Enterococcus* via infant gut models. Overall, probiotic supplementation significantly reduced antibiotic resistance gene prevalence, MDR pathogen load, and helped restore a typical early-life microbiota. However, the persistence of MDR pathogens like *Enterococcus*, with high HGT potential, highlights the need for ongoing surveillance in neonatal care. Our findings underscore the complex interactions among antibiotics, probiotics, and HGT in shaping the neonatal microbiome and support further research into probiotics for antimicrobial stewardship in preterm populations.

Developing a spatially organised in-vitro cystic fibrosis polymicrobial biofilm model with digital light processing (DLP) 3D-bioprinting.

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Abstract

Complex communities of microorganisms are a key component of the unique lung environment of people with cystic fibrosis (pwCF). These communities form heterogeneous biofilms consisting of pathogenic and commensal microorganisms. To address the disparity between in-vitro testing of antimicrobials to treat cystic fibrosis (CF) lung infections and clinical outcomes, we are developing a new 3D-printed model of CF lung biofilm infection. Clinically relevant bacteria and fungi such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus fumigatus* were incorporated into a poly (ethylene glycol) diacrylate (PEGDA) and gelatin hydrogel to form a 3D-printable structure capable of incubation for up to 72 hours at 37 °C. High throughput printing of the structures was achieved due to the customisation of an ELEGOO Mars 3 4K build plate and resin vat. Recovery of all bacterial and fungal species has been observed after printing, with comparable colony-forming units (CFUs) to the established colony biofilm model. Fluorescent confocal imaging confirmed survival and showed the spatial organisation of these microorganisms within the printed structures. RNAseq analysis was then used to determine whether gene expression profiles within the 3D-printed structure were comparable to those of these communities in natural CF lung infections and in-vitro biofilm models. Future work will utilise this biofilm printing technology to understand polymicrobial interactions, and the effect microbial spatial organisation in biofilms has on antimicrobial tolerance.

Genomic drivers of increasing intestinal colonisation with extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in rural southern Malawi

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Abstract

Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*, resistant to third-generation cephalosporins and penicillins, are prevalent in faecal and environmental samples across southern Malawi. These bacteria contribute to increased mortality among hospital patients with bloodstream infections in Blantyre, the primary urban setting from which most available sequence data originate. Limited sequence data from rural Malawi restricts our understanding of antimicrobial resistance (AMR) transmission in regions where human, animal, and environmental health intersect.

In July 2023, we collected faecal samples from 211 individuals in rural southern Malawi, with 153 of these providing follow-up samples in July 2024. Samples underwent selective culture to isolate ESBL-producing *E. coli*. The prevalence of gut colonisation with ESBL-producing *E. coli* increased from 34.0% to 54.9% ($p < 0.001$) in the cohort followed up after one year. To investigate the genomic drivers behind this rise, isolates underwent paired-end Illumina sequencing (baseline complete; follow-up in progress at time of writing).

Baseline data revealed $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}27}$ as the dominant ESBL genes, linked with a high incidence of IncF plasmids, much like urban and hospital strains from Blantyre. Genes conferring resistance to aminoglycosides, sulfonamides, tetracyclines, and trimethoprim were widespread. The globally successful ST131 lineage and ST206—rare in the hospital setting but more common in animal faeces in Malawi—were the most abundant sequence types. Follow-up sequence data will be compared to baseline data to assess colonisation transience, lineage expansion, and shifts in AMR mechanisms. Findings highlight the need for improved AMR surveillance in rural areas where treatment options are limited.

Searching for acquired antimicrobial resistance in poultry microbiomes

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Abstract

The poultry industry in Asia faces a significant threat from antimicrobial resistance (AMR) due to the extensive use of antibiotics. Effective surveillance is challenging, as antimicrobial resistance genes (ARGs) are intrinsic to many bacteria and can spread across species through horizontal gene transfer. Previously, caecal content DNA from chickens on 700 farms was analysed using 16S rDNA amplicon sequencing to examine microbial community composition (enterotypes) and AMR AmpliSeq to characterize ARG profiles (resistomes). We observed a strong correlation between enterotype and resistome, indicating that most ARGs within a resistome reflect the intrinsic resistance of the predominant bacterial species. Notably, certain samples deviated from this pattern, showing a weak enterotype-resistome correlation, suggesting potential acquired AMR.

To investigate this hypothesis, we conducted deep Illumina shotgun (n=120) and long-read Oxford Nanopore (n=56) sequencing on a subset of samples. Plasmids were identified from the assemblies and screened for ARGs, while contigs were binned into metagenome-assembled genomes (MAGs) to identify chromosomally integrated ARGs. Additionally, we assessed the role of selection pressure by comparing ARG abundance to antimicrobial residues found in meat and feathers from the same chickens.

Our findings confirm that Enterobacteria, particularly *Escherichia coli*, serve as major ARG reservoirs and highlight acquired resistance in *Campylobacter jejuni*, a notable food-borne pathogen. Geography emerged as a significant factor shaping the resistome, while the presence of plasmid-borne ARGs linked to detected antibiotic residues underscores the role of selection pressure in driving resistance. These insights emphasize the urgent need for targeted AMR mitigation strategies in the poultry industry.

From Barn to Breeze: The Journey of Antimicrobial Resistance Spread Through Air!

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Abstract

Antimicrobial resistance (AMR) represents a major global health and food security issue. If not addressed, AMR is projected to result in over 10 million deaths and an 11% annual reduction in livestock production by 2050. The agricultural use of antimicrobials further exacerbates this problem by increasing the likelihood of AMR transmission. These vital drugs are commonly employed also to treat and/ or prevent infections in animals and to control the spoilage of crops. Consequently, tackling AMR requires a "One Health" approach. Although numerous studies have examined the effects of antimicrobial use on soil, crops, wastewater facilities, and farm animals, there remains a significant knowledge gap regarding the role of air in AMR transmission in these environments. A 2021 gap analysis by the Environmental Protection Agency (EPA) identified air and aerosols as the least explored pathways for the spread of antimicrobial-resistant organisms (ARO) and antimicrobial-resistant genes (ARG). This research investigates the role of aerosols in the transmission of AROs and ARGs in the farm. Using the AirPrep™ Cub Sampler-ACD210, optimal methods for bioaerosol recovery were evaluated. This is fundamental when studying samples of low bioburden. Air sampling volume, duration and locations were compared. Sample processing using filter-washing and sonication steps were explored. Processing methods were assessed using CFU counts, DNA quantification and quality measurements and 16s-rRNA bacteria, fungal(ITS) and human(GADPH) qPCR DNA levels. The data generated will enable the ResistAMR project to perform optimal sample processing to maximize the recovery of airborne DNA for future sequencing.

Microbial Physiology, Metabolism and Molecular Biology Forum

Discovery of the early biosynthetic steps of the anticancer antibiotic pleurotin

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Abstract

Pleurotin is a secondary metabolite (SM) made by the Basidiomycota fungus *Hohenbuehelia grisea.* It was first described as an antibiotic [1] and later found to have potential as an anticancer lead due to its irreversible inhibition of the thioredoxin-thioredoxin reductase system [2]. *H. grisea* is known to make additional bioactive SMs related to pleurotin, such as the antiviral 4-hydroxypleurogrisein [3]. Total synthesis of pleurotin has been achieved, including through a stereoselective route [4], however its biosynthesis has not been characterised. In this study, we used isotope-labelled precursor feeding to show that the non-terpenoid quinone ring of pleurotin and related SMs is derived from phenylalanine. We identified putative pleurotin biosynthetic genes by combining genome sequencing of *H. grisea* through Nanopore and Illumina with comparative transcriptomics. The heterologous expression of a selected UbiA-like prenyltransferase from *H. grisea* resulted in the accumulation of the first pleurotin biosynthetic intermediate, 3-farnesyl-4-hydroxybenzoic acid [5]. This work sets the foundation to fully elucidate the biosynthesis of pleurotin and related SMs, with long-term potential to optimise their production for therapeutic use and engineer the pathway towards the biosynthesis of valuable analogues.

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Exploitation of an inducible hypermutator to rapidly identify mutants that bypass LasR- and RhlR-dependent quorum sensing control of gene expression in *P. aeruginosa.*

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen that secretes various virulence factors during infection. Many of these virulence factors are controlled by quorum sensing (QS). QS is a hierarchical system that relies on the transcription factors LasR and RhlR, which sense their corresponding autoinducer molecules, OdDHL and BHL. However, in clinical isolates, *lasR* mutants are common and, in many cases, retain virulence, indicating that QS "bypass" mechanisms exist. In principle, a better understanding of how this bypassing occurs will shed light on how QS interconnects with other mechanisms controlling virulence in *P. aeruginosa*. To further investigate these bypass mechanisms, we generated *lasR* and *rhlR* knockouts in an inducible hypermutator (HM) genetic background. In the *P. aeruginosa* HM Δ*lasR* strain, which has reduced protease secretion, we identified, among other mutations, that the transcription factor *mexT* is a hotspot for mutations that bypass *lasR* control of secreted protease production. In parallel, we aimed to develop a similar screen to look for secondary mutations that bypass RhlR-dependent gene expression. This imposes a more significant challenge because protease secretion is relatively unaffected in the *rhlR* background. Instead, we introduced *lacZ* fusions downstream of RhlR-dependent promoters, enabling easy visual assessment of bypass mutants on X-Gal. Using both screens, we have identified a number of genes for further characterization.

Direct single-molecule visualisation of DNA repair in bacteria within immune cells

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Abstract

Bacteria utilise a number of mechanisms to evade elimination from immune cells, for example, through intracellular survival inside phagocytes. One great unknown with bacterial intracellular survival is why some bacteria live, whilst other bacteria die, and what the role of DNA repair is in this process. To address this question, we explored different DNA repair pathways, namely excision repair, through proteins such as UvrB, MutM and MutY, and mismatch repair, regulated by the mismatch repair proteins such as MutL. We have developed a single-molecule tracking (SMT) method to image protein dynamics in *E. coli* after infection of macrophages. Using SMT, we have been able to track repair proteins that are either bound to DNA, indicating active repair, or freely moving, implying target searching. We show that proteins that sense, and repair, DNA damage become active in intracellular bacteria. In particular, pathways involved in correcting errors with mutagenic potential, such as the mutagenic 8oxoG lesions and mismatches, are critical. Using a novel reporter of active cell metabolism, we can correlate the level of DNA damage repair with the divergent fates of individual bacteria. We observe that metabolically active bacteria have lower DNA repair activity, indicating less DNA damage, than metabolically inactive cells. These results underline that successful repair of DNA damage is important for the survival of bacteria inside macrophages. Our novel approach is generalisable and promises novel insights into the interactions between bacteria and immune cells.

Can we Find the Universal Remote to Turn on Antibiotic Biosynthesis in Streptomyces bacteria

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Abstract

The MtrAB two-component regulatory system is highly conserved within the Actinomycetota phylum, essential for some species but not all. It is known to be an essential regulator of cell development in *Mycobacterium tuberculosis,* whilst in *Streptomyces venezuelae* it is non-essential but coordinates cell development with chloramphenicol biosynthesis. *Streptomyces* spp. produce around 50% of clinically used antibiotics, whilst >90% of their specialised metabolites remain undiscovered. Further exploration of the signalling systems regulating specialised metabolite production is crucial in addressing the global antimicrobial resistance crisis. Understanding such regulation could be key to discovering novel metabolites and optimising their production at commercially viable levels.

Our research demonstrates that *Streptomyces formicae* KY5, a biosynthetically talented strain first isolated from fungus-growing ants, employs the MtrAB system similarly to other species. *S. formicae* KY5 Δ*mtrA* mutants exhibit developmental delays, failing to sporulate under laboratory conditions, and deletion of *mtrA* increases bioactivity compared to the wild-type strain, while abolishing the production of previously studied metabolites (fasamycins and formicamycins). Co-immunoprecipitation reveals that MtrA also interacts directly with proteins encoded in over one-third of the >45 biosynthetic gene clusters in the *S. formicae* KY5 genome at the single time point sampled, suggesting MtrAB functions as a master regulator of cell development and antibiotic biosynthesis at the transcriptional and post-translational levels, even in non-model strains. We hypothesise that manipulation of MtrAB will lead to the production and identification of novel antimicrobial compounds in a wide variety of *Streptomyces* species.

Molecular basis of the biogenesis of a protein organelle for ethanolamine utilization in Salmonella

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Abstract

Many pathogenic bacteria use proteinaceous ethanolamine-utilization microcompartments (Eut BMCs) to facilitate the catabolism of ethanolamine, an abundant nutrient in the mammalian gut. The ability to metabolize ethanolamine gives pathogens a competitive edge over commensal microbiota which can drive virulence in the inflamed gut. Despite their critical functions, the molecular mechanisms governing the synthesis of Eut BMCs in bacterial cells remain elusive. Here, we report a systematic study for dissecting the molecular basis underlying Eut BMC assembly in *Salmonella*. We determined the functions of individual building proteins in the structure and function of Eut BMCs and demonstrated that EutQ plays an essential role in both cargo encapsulation and Eut BMC formation through specific association with the shell and cargo enzymes. Furthermore, our data reveal that Eut proteins can self-assemble to form cargo and shell aggregates independently *in vivo*, and that the biogenesis of Eut BMCs follows a unique "Shell-first" pathway. Cargo enzymes exhibit dynamic liquid-like organization within the Eut BMC. These discoveries provide mechanistic insights into the structure and assembly of the Eut BMC, which serves as a paradigm for membrane-less organelles. It opens up new possibilities for therapeutic interventions for infectious diseases.

NanZ sialic acid permeases form a diverse family of bacterial SLC17 transporters and share homology with lysosomal Sialin

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Abstract

Sialic acids (Sia) are ubiquitous among vertebrates as the outermost sugars on mucosal cell surfaces. N-acetyl-neuraminic acid (Neu5Ac) is the most abundant Sia in nature and the only form synthesised *de novo* by humans. Several commensal and pathogenic bacteria living in mucosal niches use specific Sia transporters to acquire host-derived Neu5Ac to boost growth and/or to modify own cell surfaces for immune evasion, enhancing their ability to colonise the host. Among the many different prokaryotic Neu5Ac transporters, Major Facilitator Superfamily (MFS) transporters are the least well-characterised at the structural-functional level. Here, we used functional expression in *Escherichia coli* to study the NanZ family of MFS Neu5Ac transporters widespread among host-associated *Bacteroidota*. First, we experimentally confirmed several uncharacterised NanZ orthologues from both within and without the *Bacteroidota*, including one from the probiotic *Verrucomicrobium*, *Akkermansia muciniphila*. Using the NanZ1 (BF1633) transporter from *Bacteroides fragilis* NCTC 93943 as a model system, we then used structural predictions and site-directed mutagenesis to identify the Neu5Ac- and proton-binding sites of this family of MFS Sia transporters, finding that NanZ proteins are SLC17 transporters homologous to the human lysosomal Neu5Ac transporter, Sialin. Finally, informed by this updated structural-functional model, we identified distant NanZ orthologues among soil *Bacteroidota*, which we showed can too transport Neu5Ac when expressed in *E. coli*. Overall, our study expands our understanding of Sia acquisition by bacteria and provides new insights into the diversity and evolution of the widespread NanZ transporters.

The sRNA Lrs1 regulates pyochelin levels in *Pseudomonas aeruginosa* PAO1-L

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Abstract

Pseudomonas aeruginosa is a major nosocomial pathogen able to colonise diverse environments. *P. aeruginosa* employs the three quorum sensing (QS) systems Las, Rhl, and Pqs to coordinate infection establishment at the population level. This is done in partnership with small non-coding RNAs (sRNAs) to quickly adapt to nutrient changes at the infection site. Here, studies on the role of sRNA Lrs1, located in the promoter region of the Pqs biosynthetic operon *pqsABCDE* are presented. Lrs1 has been previously implicated in a positive feedback loop involving the QS transcriptional regulator LasR in the strain PA14. Interestingly, we show that Lrs1 does not regulate *lasR* in the *P. aeruginosa* strain PAO1-L under the conditions tested. However, transcriptomic analysis indicated Lrs1 differentially regulates the levels of the siderophores pyochelin and pyoverdine, and that regulation is dependent on iron availability. Additionally, transcription of Lrs1 is primarily dependent on the QS systems, in both high and low iron conditions. Lrs1 conditionally affected the Pqs system only under iron depletion, which may be connected to a flow of metabolites towards central metabolism. This study identifies Lrs1 as a new post-transcriptional regulator under iron scarcity and highlights its importance in environmental adaptation in *P. aeruginosa* during infection.

How Group B streptococcus subverts intoxication by zinc and copper

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Abstract

Copper (Cu) and zinc (Zn) are mobilised by phagocytes in the host and used as potent antimicrobials to intoxicate invading bacterial pathogens. Group B streptococcus (*Streptococcus agalactiae*) is an opportunistic pathogen that causes fatal infections in neonates and survives inside macrophages as 'trojan horses' during colonisation of the reproductive tract[1]. Defined resistance mechanisms are used by streptococci and other bacteria to subvert intoxication by metals, which typically comprise efflux pumps that expel Cu[2] and Zn[3] from the bacterial cell and limit intracellular accumulation. We used a combination of transcriptomics and transposon-directed insertion sequencing to identify several novel genes that confer a survival advantage during metal intoxication[4,5], including those encoding metabolic pathways and processes that were not previously linked to Cu or Zn toxicity. Molecular characterisation of these targets has revealed a remarkable interplay between certain metabolites, their regulation or transport, and metal resistance, including the identification of compounds that inhibit, or enhance the ability of *S. agalactiae* to survive metal intoxication. These insights broaden the horizons[6] for research into mechanisms of metal ion resistance in streptococci and beyond.

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Suppressor mutations rescuing the growth of a conditionally-lethal *E. coli* mutant reveal novel regulatory links between RNase P, RNase R, and Lon protease

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Abstract

RNase P is an essential enzyme found across all domains of life that is responsible for the 5'-end maturation of precursor tRNA transcripts. Despite being the subject of hundreds of studies spanning several decades, much remains unknown about the regulation of RNase P expression and the turnover and degradation of the enzyme. In most bacteria, RNase P is a heterodimer comprised of a small protein subunit (C5 protein, encoded by *rnpA*) and an RNA subunit (M1 RNA, encoded by *rnpB*). In *E. coli*, the temperature-sensitive *rnpA49* mutation in the C5 protein subunit has arguably been one of the most well-studied mutations for examining the enzyme's activity *in vivo*. Here we report for the first time naturally-occurring temperature-resistant suppressor mutations of *E. coli* strains carrying the *rnpA49* allele. We find that *rnpA49* strains can partially compensate the temperature-sensitive defect via gene amplifications of either RNase P subunit (*rnpA49* or *rnpB*) or by the acquisition of loss-of-function mutations in Lon protease or RNase R. Our results validate previous plasmid overexpression and gene deletion complementation studies, and importantly reveal novel links between *E. coli* RNase P and the regulatory pathways involving RNase R and Lon protease. Specifically, the latter implicates the mutant C5 protein subunit is likely a target for Lon proteolysis, potentially adding it to the list of bacterial RNA-binding proteins regulated by Lon-mediated quality control mechanisms. This discovery opens exciting new avenues for future investigations into RNase P regulation and metabolism in *E. coli*.

Assessing the specificity of metallochaperone required for the assembly of the Neisseria nitrite reductase, AniA

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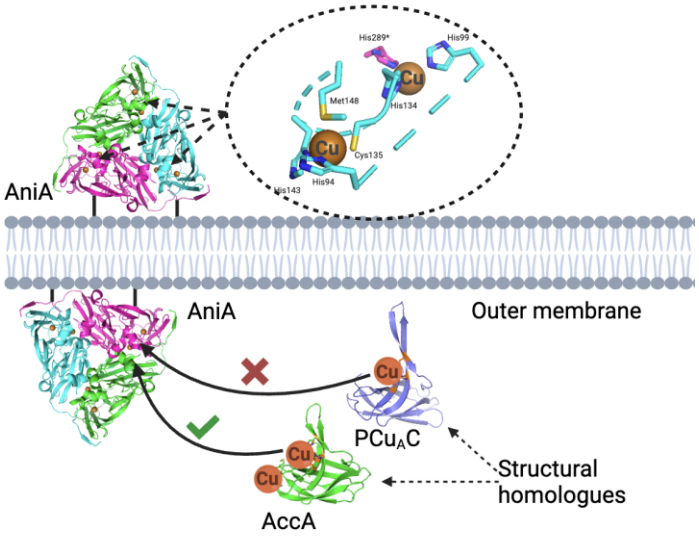
Abstract

Neisseria gonorrhoeae infects the human genitourinary tract and causes the sexually transmitted infection gonorrhoea. In this O2-limited host niche, *N. gonorrhoeae* relies on nitrite as the terminal electron acceptor in anaerobic respiration. In this process, nitrite is reduced to nitric oxide by the copper-dependent nitrite reductase, AniA.

AniA exists on either side of the outer membrane and is a target for vaccines and novel antimicrobials. Copper insertion into the AniA active site, and thus enzyme activity, requires a periplasmic copper-binding accessory protein AccA. AccA is part of a large family of periplasmic copper-binding proteins. Here we hypothesised that the relationship between AccA and AniA is specific. To test this hypothesis, AccA was replaced with the PCu_AC homologue from *Thermus thermophilus*, creating an *ΔaccA/pCu$_A$C*$^+$ mutant strain. Microaerobic cultures of this mutant did not grow or consume nitrite, but, both were rescued upon the addition of Cu salt. These results suggests that in the presence of PCu_AC but absence of AccA, AniA does not acquire copper and is inactive, unless additional copper is supplied. Therefore, PCu_AC does not functionally replace AccA in cells. Biochemical studies using purified proteins further showed that copper transfer to AniA from PCu_AC is 15X slower than from AccA.

Our study indicates that the interaction between AccA and AniA is specific and favourable for the transfer of copper. This specificity suggests the feasibility of targeting the AccA-AniA interactions for novel antimicrobials against multi-drug-resistant *N. gonorrhoeae*.



Targeting Metal Homeostasis: The Antimicrobial Potential of Chelant X

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Abstract

Metal chelating agents have emerged as promising tools for combating antimicrobial resistance (AMR) due to their ability to disrupt microbial metal homeostasis. This study investigates the antimicrobial potential of Chelant X against *Escherichia coli*, *Serratia marcescens*, and *Staphylococcus aureus*, exploring its mechanism of action through metal ion manipulation. Inductively coupled plasma mass spectrometry (ICP-MS) revealed that Chelant X reduced iron levels and increased manganese in *E. coli* and *S. marcescens*. Similarly, quantitative PCR and proteomic analyses carried out in parallel identified upregulation of iron uptake genes, especially the iron-enterobactin import system, consistent with a response to iron starvation. In *S. aureus*, Chelant X exhibited a biphasic effect: low concentrations increased iron levels, while higher doses reduced both iron and manganese while elevating copper levels. qPCR showed upregulation of the copper exporter *copA* at these higher concentrations. Checkerboard assays using Chelant X indicated antagonistic interactions with both iron and manganese in *E. coli*, but synergistic effects with iron or copper in *S. aureus*. These results suggest that Chelant X disrupts metal ion homeostasis, with concentration-dependent shifts from iron toxicity to copper toxicity in Gram-positive bacteria. By targeting essential metals that are critical for microbial survival, Chelant X offers a novel approach to overcome AMR. Further research into chelating agents as a strategy for metal ion toxicity is warranted to develop new treatments against resistant pathogens.

Mixed signals: The influence of nutrient co-limitation on diatom phosphorus sensing and acquisition mechanisms

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Abstract

Diatoms are a major component of the marine phytoplankton community, and account for 20% of global primary productivity. The marine environment fluctuates greatly across a range of spatiotemporal scales, especially in regard to nutrient availability. Two nutrients that are crucial for diatom growth are nitrogen (N) and phosphorus (P), the distribution of N and P is influenced by a number of abiotic and biotic factors, and is changing due to anthropogenic activities. In many oceanic regions diatoms experience deprivation of N and P simultaneously, and yet how they cope with co-limitation is poorly understood. To be successful, diatoms must also be able to sense and rapidly respond to transient nutrient pulses in their environment.

Previous work has shown that P-starved *Phaeodactylum tricornutum* cells show rapid Ca^{2+} elevations when resupplied with P, with the Ca^{2+} acting as a messenger that can trigger cellular recovery from P limitation. One of the earliest metabolic adaptations identified following P resupply is an increase in N uptake and assimilation, suggesting rapid cross-talk between P signalling and N metabolism. To further characterise the interplay between N and P in diatoms, we have investigated how co-limitation by these nutrients alters P sensing and starvation responses in two marine diatoms. We identify distinct physiological adaptations are induced under N and P co-limitation compared to those observed under single nutrient limiting conditions. Our data suggests sophisticated regulatory mechanisms allow diatoms to balance N and P status within the cell, enabling the prioritisation of limiting resources when nutrients are scarce.

Cyclic di-GMP modulates virulence behaviours in multidrug resistant clinical isolates of *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii is listed as a critical priority pathogen by the World Health Organisation. It is able to form strong biofilms which are key to its pathogenic success enabling it to tolerate environmental aggressions, including antimicrobial treatments. While its antibiotic resistance mechanisms are well-established, comparatively little is known about how *A. baumannii* regulates biofilm formation and virulence. Here, we explore the role of the second messenger cyclic di-GMP (cdGMP) in regulating biofilm and virulence-related behaviours in multidrug resistant clinical isolates of *A. baumannii*. For this, we created genetic constructs that enabled us to artificially manipulate internal cdGMP levels, which we validated using a bespoke fluorescent cdGMP reporter. To uncover the cdGMP regulon in *A. baumannii*, we performed a differential RNA sequencing experiment comparing high versus low cdGMP levels in clinical isolates. This analysis revealed that cdGMP upregulates genes involved in surface attachment and exopolysaccharide production, while concomitantly causing the downregulation of genes associated with motility. We confirmed the consequences of these transcriptional changes through phenotypic validation. Furthermore, we established that overall pathogenicity was substantially impacted by high levels of cdGMP, as these attenuated *A. baumannii* virulence *in vivo*. To elucidate the native elements driving cdGMP oscillations, we performed a genomic analysis on the conservation of the enzymes that control cdGMP intracellular levels (diguanylate cyclases and phosphodiesteresases) throughout the *A. baumannii* phylogeny using a customised pangenome of >9,600 genomes, ultimately uncovering which enzymes were more closely associated with virulence. These findings shed new light on virulence regulation in this critical-priority pathogen.

RecA filament kinetics explain heterogenous SOS response induction and cell death in *E. coli*

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Abstract

The SOS response is a widely conserved bacterial stress response, responsible for regulating both high-fidelity and mutagenic DNA repair amidst many stress tolerance functions. It is well-known that single cells spontaneously induce the SOS response during normal growth and that SOS levels are highly variable across cells in a population, but the root cause and potential function of this heterogeneity remain unknown. Canonically, induction proceeds due to processing of DNA damage to form a RecA-single stranded DNA filament, which prompts self-cleavage and degradation of LexA, the regulon's master transcriptional repressor. We imaged fluorescent labelled RecA in single *E. coli* cells without DNA damaging treatment using mother machine microfluidics. We uncovered that foci indicative of RecA filament events precede spontaneous, transient SOS response induction and that the duration of RecA filaments modulate SOS response heterogeneity. We find that genetic perturbations affecting RecA filament stability and LexA degradation influence RecA structures and gene induction kinetics, and that RecA filament duration predicts cell death events. Taken together, our results show that spontaneous SOS induction, and its heterogeneous magnitude, is in fact a precise response of single cells to prevent death from endogeneous DNA damage.

Trace metal availability drives microbial metabolic shifts and shapes community functional diversity

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Abstract

Microbial life relies on redox reactions to power essential cellular and metabolic processes, in which oxidoreductases play a central role. These enzymes use trace elements, particularly transition metals, as cofactors that are crucial for controlling electron flow between donors and acceptors, and contribute to maintaining the redox balance and thermodynamic disequilibria of natural systems. Despite their low abundance in the environment, trace metals distribution and availability might play a key role in influencing microbial functional diversity, shaping microbial community composition, and modulating biogeochemical cycling processes.

Here, we report findings from both field and laboratory experiments elucidating the impact of trace elements availability on microbial functional diversity. Metagenomic data obtained from environmental samples suggest that trace metals availability shapes the diversity of the oxidoreductases in microbial communities, driving functional diversity in natural ecosystems. Furthermore, combining physiological, proteomic, and functional analyses on microbial model organisms, we show that trace metal deficiency imposes energetic costs on microbial growth, delays microbial respiration transitions, alters electron acceptor utilization order, and enhances metal scavenging mechanisms. Our findings provide valuable insights into how trace metals regulate microbial functions, offering new perspectives for optimizing microbial processes in environmental and industrial applications.

Progress Towards Understanding the Function of the Essential WhiB-like Regulator 'WblE' in *Streptomyces* spp.

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Abstract

The WhiB-like (Wbl) family of transcriptional regulators are phyletically confined to the Actinobacteria, where they act as global transcription factors controlling vital physiological processes. Wbl proteins are characterised by their unique C-terminal DNA-binding motifs and invariant cysteine residues which ligate oxygen and nitric oxide (NO) sensitive [4Fe-4S] clusters. Interactions between Wbl proteins and the primary RNA polymerase sigma factor protect the cluster from oxygen- but not NO-based degradation *in vitro,* alluding to their putative role as specific NO sensors.

The genus *Streptomyces* exhibits a talent for the de novo synthesis of bioactive secondary metabolites, which is intimately coordinated with their complex development, wherein Wbl proteins play crucial but enigmatic roles. Notably, the *Streptomyces wblE* gene encodes a seldom studied WhiB-like regulator of unknown function, homologous to the essential WhiB1 regulator in *M. tuberculosis*. Phylogenetic analysis implies that WblE and its orthologues are contemporary representatives of an ancient Wbl ancestor, undergoing expansion within the Actinobacteria and hence provides an important model for all Wbl proteins.

In this work an engineered CRISPR-Cas9 system was used to generate conditional knockdowns of *wblE in vivo* and demonstrate its essentiality in *S. venezuelae*. Moreover, ChIP-Seq revealed that, akin to other Wbl proteins, WblE functions as a global transcription factor, sharing ancestral targets with *M. tuberculosis* WhiB1 and, importantly, binding the promoters of a range of essential genes. This work also presents the purification and *in vitro* analysis of WblE alone and in complex with the primary σ-factor, as well progress investigating several novel interaction partners.

Harnessing epigenetic and metabolic features of *Lactiplantibacillus plantarum* for potential targeted immunomodulatory probiotics.

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Abstract

Gut microbes play a vital role in maintaining homeostasis through continuous interaction with our mucosal immune system. Yet, the key commensal microbes that restore or protect health from disease remain unclear. This lack of clarity limits the development of effective microbial therapies, including probiotic treatments, as the molecular mechanisms underlying their immunomodulatory properties remain unclear. We recently reported that certain strains of the probiotic *Lactiplantibacillus plantarum* (LP) can enhance macrophage interaction due to its prominent self-aggregating ability, leading to increased bacteria internalization triggering elevated production of type I interferon (IFN-I) cytokines via DNA sensing that are essential to combat microbial infections and autoimmune disorders. Furthermore, using fluorescence assays, we now know that internalisation of such massive aggregates disrupts the lysosomal membrane, causing phagolysosome destabilization and subsequent leakage of bacterial contents including DNA into the cytosol, thereby activating intracellular sensing. Additionally, RNA sequencing of LP showed upregulation of genes indicating a preference for specific monosaccharides, which when cultured in, enhanced its growth, lowering the pH of the media promoting self-aggregation behaviour critical to these immune-activating interactions. Interestingly, this high self-aggregation profile correlated positively with low levels of bacteria DNA methylation that were also dependent on the monosaccharide metabolised. Thus, our data suggest that self-aggregation and DNA methylation are two key physicochemical features of LP, linked to its sugar metabolism that define its immunomodulatory properties. Elucidating these interconnected mechanisms could be important to generate targeted probiotic formulations with specific strains and sugars, offering novel immunomodulatory approaches against disorders affecting the mucosa.

Microbial Warfare: conflicts between species, strains, and mobile genetic elements

Invited: “All the World’s a Phage” - Exploring phage-host interactions

Tim Blower ORCID iD

Durham University, Durham, United Kingdom. New England Biolabs, Ipswich, USA

Abstract

Bacteriophages (phages) outnumber bacteria by ten to one, with an estimated 10^30 phages causing infections at a rate of 10^25 a second. This huge selection pressure has led to the evolution of bacterial defence systems that protect from phage predation. Many of these defence systems have already proven useful to biochemists; the restriction-modification and CRISPR-Cas systems underpin the recombinant DNA and genome editing revolutions. Defence systems are commonly found grouped into “islands”, but functionality has not previously been systematically tested. Using phages isolated by Durham undergraduates we are exploring the complementary biochemistry of a range of defence systems in islands, including BREX, Type IV restriction, and abortive infection. Understanding interactions between phages and their hosts opens up new routes for the control of bacterial behaviour, and identifies potential tools for biotechnology.

Invited: The contribution of interactions between mobile elements to the bacterial pan-genome

Eduardo Rocha ORCID iD

Institut Pasteur, Paris, France. CNRS, Paris, France

Abstract

Horizontal gene transfer driven by phages or conjugative elements allows the acquisition of complex adaptive traits and their transmission to subsequent generations. This speeds up evolutionary processes as exemplified by the acquisition of virulence traits in emerging infectious agents and by antibiotic resistance in many human pathogens. I'll describe how differences between mobile genetic elements in terms of their mechanism of vertical and horizontal transmission result in diverse patterns of gene transmission. These patterns are further modified by the interactions between mobile genetic elements within cells. As a result, the changes in pangenomes driven by horizontal transfer can have a multitude of causes and be subject to very diverse selective pressures.

Invited: Conflicts between plasmids and with defence systems drive the evolution of AMR plasmid transmission

Tatiana Dimitriu ORCID iD

University of St Andrews, St Andrews, United Kingdom

Abstract

In bacteria, genes conferring antibiotic resistance are mostly carried on conjugative plasmids, mobile elements which spread horizontally among bacterial hosts by conjugation. Here I will discuss experimental work in which we show that plasmid conjugation rate, levels of AMR conferred by plasmids and AMR gene content can all change rapidly depending on the ecological opportunities for plasmid transmission, with consequences for the spread of antibiotic resistance.

Invited: Discovery and characterisation of toxin-antitoxins and other phage defence systems

Gemma Atkinson ORCID iD

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Abstract

Toxin-antitoxin (TA) systems are ubiquitous, diverse and highly mobile gene pairs of microbes. They consist of a gene encoding a a toxin that dramatically inhibits bacterial growth and an adjacent gene encoding an antitoxin that protects against, and neutralises the toxic effect. In addition to the two gene TA systems, there are also single gene TAs where the toxin and antitoxin are fused and expressed as a single protein, and three gene systems where the TA genes are accompanied by a gene encoding a SecB-like chaperone that stabilises the antitoxin (toxin-antitoxin-chaperone TAC systems). We have recently identified fused TA (CapRel) and TAC (HigBAC and CmdTAC) systems that are encoded on prophages in Escherichia coli genomes, and function as innate immune systems, protecting against infection by incoming phages. As variable regions of prophages are rich seams of novel biology, we are mining these regions with high-throughput bioinformatics methods to discover new microbial immune systems that we then validate experimentally.

A good defence is a bad offense: CRISPR-Cas in inter-plasmid competition

David Sünderhauf ORCID iD[1], Jahn Ringger[2], William Gaze[1], Stineke van Houte[1]

[1]University of Exeter, Penryn, United Kingdom. [2]University of Basel, Basel, Switzerland

Abstract

CRISPR-Cas is a prokaryotic immune system that defends bacterial hosts from mobile genetic elements. When carried on plasmids, CRISPR-Cas contributes to plasmid competition – but it is unclear under which conditions a plasmid benefits from carrying this immune system. Plasmid-encoded CRISPR-Cas acts defensively when it protects the plasmid carrying it from displacement by a competitor plasmid. Alternatively, plasmid-encoded CRISPR-Cas acts offensively when the plasmid carrying it invades a new host where it displaces a competitor. Toxin-antitoxin (TA) systems are near ubiquitous on large plasmids, and typically function as addiction systems that prevent plasmid loss.

Here, we investigate the relative benefits of a plasmid-encoded CRISPR-Cas system when adopting an offensive or defensive function against a competitor plasmid with or without a TA system. We conjugatively mated three *Escherichia coli* host strains; one host carried RP4 which encodes TA operon *parABCDE*. The second host carried pKJK5::csg which encodes CRISPR-Cas9, programmed to target RP4. The final host remained plasmid-free. In this way, we could test CRISPR-Cas under defensive and offensive conditions while tweaking CRISPR-Cas9 and *parABCDE* activity on or off.

Acting defensively, CRISPR-Cas was effective at preventing RP4 invasion of pKJK5 hosts. In offense however, CRISPR-Cas was detrimental to pKJK5 when *par* TA was active on competitor plasmid RP4 and prevented invasion of RP4+ cells by pKJK5. This work reveals the limitations of a plasmid-based CRISPR-Cas system: its primary role is defensive rather than offensive. This has implications on the distribution of prokaryotic defences, and the utility of CRISPR-Cas carried by highly mobile plasmids.

Multi-conflict islands are a widespread trend within *Serratia* spp

Thomas Cummins[1], Surajpal Songra[1], Stephen R Garrett[2], Tim R Blower[3], Giuseppina Mariano[4]

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Abstract

Bacteria carry numerous anti-phage systems in 'defence islands' or hotspots. Recent studies have delineated the content and boundaries of these islands in various species, revealing instances of islands that encode additional factors, including antibiotic resistance, stress genes, Type VI Secretion System (T6SS)-dependent effectors, and virulence factors.

Our study identifies three defence islands in the *Serratia* genus with a mixed cargo of anti- phage systems, virulence factors and different types of anti-bacterial modules, revealing a widespread trend of co-accumulation that extends beyond T6SS-dependent effectors to colicins and contact-dependent inhibition systems. We report the identification of four distinct anti-phage system/subtypes, including a previously unreported Toll/IL-1 receptor (TIR)- domain-containing system with population-wide immunity, and two loci co-opting a predicted T6SS-related protein for phage defence. This study enhances our understanding of the protein domains that can be co-opted for phage defence and of the diverse combinations in which known anti-phage proteins can be assembled, resulting in a highly diversified anti-phage arsenal.

Genomic analyses to infer defence system interactions among global Pseudomonas aeruginosa populations

Charlotte Chong[1], Aaron Weinmann[2,3,4,5], Aleksei Agapov[6], Jo Fothergill[7], Michael Brockhurst[8], Julian Parkhill[5], R. Andres Floto[2,3,9,10,11], Mark Szczelkun[12], Edze Westra[6], Kate Baker[1]

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Abstract

The evolutionary arms race between bacteria and phages has driven the development of complex anti-phage defence systems. Many recent discoveries have revealed that microbial genomes are densely packed with a diverse array of defence systems. Emerging evidence suggests that bacterial defence systems (DSs) can interact both synergistically and antagonistically. Ecological studies that draw out over-representations of the co-occurrence and avoidance of DSs in naturally evolving bacterial populations might point to interactions with an increased likelihood of having genuine biological relationships. *Pseudomonas aeruginosa* is an ideal organism in which to explore these interactions as it has a highly plastic genome, is contains many DSs, and evolves across various ecological niches. Here, we analysed the interactions among DSs, and other accessory genome elements (n=512), in a recently curated dataset of 4,289 *P. aeruginosa* genomes. This revealed differences in DS content, antimicrobial resistance (AMR) determinants, phages and other mobile genetic elements (MGEs) according to ecological niche. Co-incidence analyses revealed multiple phylogenetically independent associations (n=426) and disassociations (n=50) among DSs and other MGEs. We further explored colocalization and explanations for interaction by niche. Ultimately, this work revealed that DSs and their interactions vary across the landscape of *P. aeruginosa* population and that significant interactions among DSs and other accessory genome elements exist, which may indicate mechanistic synergy or antagonism. Understanding these patterns of DS interactions and their variation across ecological niches is crucial, as it sheds light on the dynamics of horizontal gene transfer, the spread of AMR, and evolutionary pressures shaping bacterial genomes.

Gene Transfer Agents: Domesticated Viruses as Vehicles of Horizontal Gene Transfer

Matt Craske ORCID iD

University of York, York, United Kingdom

Abstract

Bacterial gene transfer agents (GTAs) are viewed as domesticated bacteriophage-like entities that have lost the ability to selfishly self-replicate their genome. Instead, GTAs exclusively package and transfer random fragments of a host bacterial genome to closely related cells. The transduction-like mechanism utilised by GTAs results in cell lysis of a producing cell and, in turn, sacrifices a subset of the total population. The long-term maintenance of these elements, despite their associated fitness costs, has prompted further questions on their influence on bacterial evolution. Currently, the ecological benefit of GTA production remains largely unknown. Here, we use ChIP-seq and RNA-seq to further understand the wider regulatory effects of the GTA transcriptional activator, GafA, and repressor, *rcc00280*, within the model GTA producing organism, *Rhodobacter capsulatus*. These data provide further insights of the functional relevance of GTAs when the host is faced with alternative carbon sources, antibiotics and other ecological conditions. We demonstrate that different classes of antibiotics can induce or repress GTA production, which may have wider implications on the spread of antibiotic resistance genes in the environment or influence treatment. Meanwhile, *R. capsulatus* GTA overproduction increases cell survival in the presence of DNA damaging agents, such as zeocin, consistent with previous observations in *Caulobacter crescentus*. Our study makes important steps toward deciphering the function of GTAs and the environmental conditions under which they are beneficial to the host.

The mechanism and evolution of the Phage Growth Limitation (Pgl) system

John Bruce ORCID iD, Paul Hoskisson

University of Strathclyde, Glasgow, United Kingdom

Abstract

Bacterial defence systems are mechanistically diverse but follow an established pattern: a cell is infected; a defence mechanism prevents phage replication; and the cell survives to reproduce, or in dying prevents amplification of phage that could then infect other cells. However, members of the multicellular bacterial genus *Streptomyces* carry a unique viral defence system that fails to prevent phage replication upon initial infection. The phage growth limitation (Pgl) system, while unable to prevent phage replication and host lysis, appears to 'tag' the DNA of phage produced during the initial infection. When these 'tagged' phage subsequently infect a Pgl^+ host they are recognised and destroyed but can replicate and lyse hosts lacking the Pgl system (Pgl^-). Here, we will present recent work on how the Pgl system senses and responds to infection; who benefits given initial infections require host lysis; the costs of PGL mediated-defence and how these are mitigated; and if, by producing viruses that kill Pgl^- competitors, the Pgl defence system can also act as a weapon.

To kill or not to kill: deciphering the mechanisms promoting activity of a facultative algicidal marine bacterium.

Courtney Swink ORCID iD[1,2], Adam Monier[2], Glen Wheeler[1], Nick Smirnoff[2], Michael Cunliffe[1], Katherine Helliwell[1,2]

[1]Marine Biological Association, Plymouth, United Kingdom. [2]University of Exeter, Exeter, United Kingdom

Abstract

Diatoms are important primary producers in the ocean, but they do not live in isolation and interactions with other members of the microbial community can have significant impacts on their growth and productivity. A facultative algicidal bacterium, *Ponticoccus alexandrii,* has been recently isolated from the Western English Channel and kills diatoms in a species-specific manner. The molecular mechanisms mediating pathogenicity of algicidal bacteria towards diatoms are poorly understood and we intend to investigate this using *P. alexandrii* as a new model system. Pre-exposure to diatom necromass promotes an algicidal phenotype in *P. alexandrii* while pre-growth on nutrient rich medium induces a mutualist phenotype in the bacteria when co-cultured with the diatom. Microscopy image analysis revealed the algicidal phenotype induces bacterial attachment to diatom prey. Moreover, this attachment precedes diatom chlorophyll intensity decline compared to the non-algicidal control, suggesting physical contact with the diatom is necessary for algicidal activity. Comparative genomics analysis enabled examination of the genetic attributes of *P. alexandrii* in relation to other algicidal members of the *Roseobacter* lineage. This analysis suggests the potential importance of plasmid genomic units alongside genes for quorum sensing, chemotaxis, attachment as well as a type IV secretion system in this microbial interaction. Further work aims to characterize candidate pathogenicity genes using a newly established genetic transformation system for *P. alexandrii* providing a deeper understanding of the mechanisms governing such interactions and their broader prevalence in the marine environment.

Phage vs. Phage: When Pseudomonas' own prophages step up the defence

Anna Olina ORCID iD[1], Aleksei Agapov[1], Xinyan Yu[2], Stineke van Houte[1], Edze Westra[1]

[1]University of Exeter, Penryn, United Kingdom. [2]Nanjing Medical University, Nanjing, China

Abstract

Phages, the most abundant biological entities on Earth, shape microbial evolution and influence the composition and function of microbial communities. Recent studies reveal that bacteria dedicate substantial genome space to antiphage defences. While defence genes are often found on prophages (phages integrated into the bacterial genome), systematic studies on their role in bacterial immunity are lacking.

This study investigates prophage-encoded defence systems in bacterial resistance against both lytic and temperate phages, using *Pseudomonas aeruginosa* as a model. From 500 clinical isolates, we isolated temperate phages and generated lysogens in a "defenceless" PAO1 mutant strain lacking prophages and defence systems. Challenging these lysogens with 60 lytic phages revealed that more than half confer resistance, ranging from resistance to only a few phages to nearly one-third of all tested.

To uncover underlying defence mechanisms, we integrate experimental data with bioinformatics. We first generate knockouts of prophage-encoded defence genes to assess their impact on resistance phenotypes. Absorption assays help distinguish between surface-modification defences and those acting at later infection stages. Sequencing of prophages shows that resistance patterns often align with phylogeny, where closely related prophages frequently, but not always, provide similar resistance.

While studies of bacterial-phage coevolution often focus on single host-virus pairs, our work explores interactions among bacteria, temperate phages, and lytic phages. This added complexity profoundly influences infection dynamics, suggesting that interactions among diverse phage types are critical in shaping microbial population dynamics and genome evolution.

Microbes evolve individual and collective defences against common T6SS toxins

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[1]University of Manchester, Manchester, United Kingdom. [2]University of Basel, Basel, Switzerland

Abstract

In competitive communities, microbes face attacks by rivals armed with toxic weaponry, including diverse and widespread Type 6 Secretion Systems (T6SSs). T6SS attacks can in turn select for both individual- and collective resistance mechanisms, transforming the outcomes of microbial warfare. Despite their importance, however, we have a poor understanding of how these different scales of anti-competitor defence evolve. To address this, we combine genomics, confocal microscopy and colorimetric assays to scrutinise diverse resistance mechanisms against common T6SS toxin types. By quantifying T6SS killing at both cell- and population lengthscales, we delineate between individual-scale resistance mechanisms, which protect cells in isolation, and collective resistance mechanisms, where emergent group behaviours lead to improved survival. Using whole-genome sequencing, and by comparison with single-gene-knockout (Keio) strains, we determine the genetic bases of different resistance phenotypes, and profile their emergence over evolutionary timescales using Pool-Seq. We conclude that "T6SS resistance" is actually a multifaceted phenomenon, incorporating multiple scales of defence in a manner dependent on T6SS toxin type. We also show how different resistance mechanisms in turn impact the specificity and spectrum of protection against T6SSs and other hazards, including antimicrobials, detergents and bacteriophages. By exploring the emergence of different T6SS resistance mechanisms, and their scales of effect, our work helps us to understand how toxin warfare shapes microbial ecology and evolution.

Sex, HGT and symbioses: genetic recombination across the microbial eukaryotic tree of life

Invited: Rethinking eukaryotic genome innovation: New perspectives on the prevalence, integration and implications of horizontally transferred genes

Jolien van Hooff ORCID iD

Wageningen University & Research, Wageningen, Netherlands

Abstract

While eukaryotic horizontal gene transfer (HGT) was long considered rare, as more genomes become available, HGT appears to be considerably implicated in genome innovation in diverse eukaryotic lineages. Yet, we know still very little about eukaryotic HGT. A recent review indicated that eukaryotic microbes obtained about 1% of their genes through HGT. However, this estimate mainly relies on studies that did not explore more ancient HGTs or eukaryote-to-eukaryote transfers, highlighting the need for more extensive investigations into eukaryotic HGT. Such analyses help refine our impact estimate, and allow us to broadly characterise eukaryotic HGT. The latter will assist in identifying its key determinants. Here, I highlight HGT patterns in eukaryotic microbial clades - including old and young, prokaryote- and eukaryote-derived HGTs. Our work suggests that, indeed, extant lineages may have more than 1% of their genomes stemming from HGTs, yet they are outnumbered by gene duplications. In some taxa, younger HGT genes localise in regions other than older ones, i.e., typically gene-sparse regions. If retained, HGT genes might thus move to gene-denser regions, which I hypothesise comes along with more stable gene expression. Moreover, I illustrate that HGT might not be limited to affecting a species' metabolic arsenal, but may have wider functional implications. Altogether, given its putative impact, future research should focus on finding out why certain species, or maybe even certain genomic regions within a species, are more prone to acquiring foreign genes, as this will shed light on the constraints and mechanisms at play.

Invited: The impact of horizontal gene transfer in the acquisition of plastids, the emergent model *Rapaza viridis*.

Ivan Garcia-Cunchillos ORCID iD[1], Yuichiro Kashiyama ORCID iD[2], Anna Karnkowska ORCID iD[1]

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Abstract

Theoretical models approaching the integration of plastids in the eukaryotic cell predict that multiple organisms, either prokaryotic and eukaryotic, prey or endosymbionts, provide the host nucleus with genes by horizontal gene transfer previous to the fixation of the permanent plastid. Thus, while the ultimate plastid donor is a single organism, the complete set of genes coding plastid-targeted proteins could proceed from diverse sources. However, the ancient origin of these events of plastid acquisition is an obstacle to studying the impact and the ancestry of these horizontal gene transfers. *Rapaza viridis* is a mixotrophic euglenid exhibiting obligatory kleptoplasty on a specific green alga, *Tetraselmis* sp., and temporarily controls the kleptoplast activity, taking advantage of the photosynthetic products. Experimental evidence demonstrated that *Rapaza viridis* possesses a gene encoding nitrate reductases in its genome, and its expression is fundamental in establishing this kleptoplasty. Remarkably, phylogenetic analyses of this gene revealed its acquisition by a horizontal gene transfer event. Despite the apparent exceptionality of such an event, the genome of *Rapaza viridis* harbors numerous genes coding kleptoplast-targeted proteins involved in several plastid metabolic pathways. Moreover, the origin of these genes is diverse, and multiple donors, presumably previous endosymbionts or algal prey, seem to have contributed to this mosaic genetic pool. Consequently, *Rapaza viridis* represents an emergent model of plastid integration and provides an exceptional opportunity to study the magnitude of horizontal gene transfer in establishing organelles in the eukaryotic cell.

Invited: Genetic screens in a malaria parasite provide a window on sexual reproduction in a divergent eukaryote

Oliver Billker ORCID iD

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Abstract

Malaria is caused by protozoan parasites of the genus Plasmodium, which is transmitted between hosts by a mosquito vector, causing more than half a million deaths annually, with 70% of deaths occurring in African children under the age of seven. Malaria parasites are divergent eukaryotes with a complex life cycle, whose asexual replication in erythrocytes is responsible for disease, but whose transmission by mosquitoes depends entirely on sexual reproduction.

In a tractable rodent model, we have scaled up the targeted disruption of parasite genes to the point of enabling genome scale genetic screens at different life cycle stages. This has revealed how gene essentiality has evolved differently in different parts of the genome. It has also allowed us map how parasite metabolism is reorganised as the parasite moves between different hosts and tissues, exposing changing drug vulnerabilities throughout the life cycle.

Sexual reproduction is essential for malaria parasites to infect mosquitoes, and our screens identify hundreds of parasite genes involved in the process. An analysis of fertility genes leads us to propose potential targets for transmission blocking interventions among some of the unique aspects of parasite sex. It also reveals unexpected, conserved aspects of developmental regulation, sperm biogenesis and gamete fusion that may represent ancestral mechanisms present already close to the last eukaryotic common ancestor.

Invited: Some molecular perspectives on a nascent endosymbiosis

Tom Richards

University of Oxford, Oxford, United Kingdom

Abstract

Endosymbiosis was a key factor in the evolution of eukaryotic cellular complexity. Yet the mechanisms that allow host regulation and maintenance of intracellular symbionts, a pre-requisite for long-term associations and therefore organelle evolution, are largely unknown. We have been [further] developing the protist (ciliate) Paramecium bursaria as a model system for studying stable endosymbiotic interactions. I will describe an immune-like glycan-sensing network, partly assembled through horizontal gene-transfers (HGTs), that enables the P. bursaria to control its algal endosymbionts. Using phylogenetics, RNA-interference (RNAi), and metabolite exposure experiments, we show that P. bursaria regulates endosymbiont destruction using glycan-sensing and processing. This system includes a eukaryotic-wide chitin-binding chitinase-like protein (CLP) localised to the host phago-lysosome. RNAi of CLP alters expression of eight glycan-processing genes, including two prokaryote-derived HGTs, during endosymbiont destruction. Furthermore, glycan-sensing/processing dynamically regulates endosymbiont number in P. bursaria, providing plasticity crucial to maximize host fitness across variant ecological conditions. CLP is homologous to a human phagocyte-associated innate immune factor, revealing how immune functions can be alternatively adapted and expanded -partly through HGT- for endosymbiont control.

Chromosome divergence and hybrid sterility in budding yeasts

Jasmine Ono ORCID iD[1], G. Ozan Bozdag ORCID iD[2], David Rogers ORCID iD[3], Duncan Greig ORCID iD[4]

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Abstract

New species are formed when they become reproductively isolated from one another, usually as a consequence of a combination of isolating barriers. We investigated the potential genetic causes of reproductive isolation between two closely related species of budding yeast, Saccharomyces cerevisiae and S. paradoxus. F1 crosses between these species are viable but infertile, with 99% of gametes failing to survive. We find that the primary reproductive barrier is chromosomal divergence, which results in mis-segregation of chromosomes during meiosis due to the rejection of recombination. All chromosomes in Saccharomyces yeasts are essential, so a gamete missing a single chromosome is inviable. In this system, a lack of recombination is the cause of hybrid sterility. We then break this chromosomal species barrier by restoring recombination in the hybrid, allowing us to uncover evidence of genic incompatibilities between diverged alleles from the two parental species.

Tackling AMR through multidisciplinary and cross-sector collaboration

Futures AMR Network: Building the next generation of AMR leaders.

Linda Oyama ORCID iD[1], Mojgan Rabiey ORCID iD[2], Nikhil Bhalla ORCID iD[3], Katie Lawther ORCID iD[1], Alberto Longo ORCID iD[1], Janice Spencer[4], Prachi Bendale[1]

[1]Queen's University Belfast, Belfast, United Kingdom. [2]University of Warwick, Warwick, United Kingdom. [3]Ulster University, Belfast, United Kingdom. [4]Glasgow Caledonian University, Glasgow, United Kingdom

Abstract

Addressing antimicrobial resistance (AMR) requires both short- and long-term expertise, dynamic solutions, and active engagement with tomorrow's leaders - early career researchers (ECRs). The Futures AMR Network (FAN) is a vibrant, transdisciplinary community dedicated to tackling the complexities of AMR by connecting and developing talented individuals from diverse sectors at the early stages of their careers. Led by outstanding UK ECRs and supported by world-leading AMR experts, FAN's vision is to harness young talent for innovative solutions to the AMR challenge.

FAN integrates expertise from diverse fields such as behavioural economics, life sciences, chemistry, social sciences, engineering, and the arts. By fostering collaboration across disciplines, engaging stakeholders, and connecting with the public, FAN aims to comprehensively address the multifaceted challenges of tackling AMR. ECRs are pivotal innovators who bring fresh perspectives, enthusiasm, openness to innovation and ground-breaking ideas. They leverage modern technologies, push boundaries, and drive innovation and advancements in AMR research through modern technologies.

FAN creates an inclusive, equitable platform for UK ECR AMR community to tackle systemic inequalities, contribute innovative solutions, and revitalize AMR discussions. By empowering, equipping and harnessing these young talent and future leaders, FAN will advance understanding, generates new ideas, and accelerates discoveries to combat AMR.

In this section, we will outline FAN's vision, highlighting opportunities to join its dynamic membership or partner with us to shape the next generation of AMR thought leaders. Together, through collaboration and evidence-based working groups, we can make a meaningful impact in the battle against AMR.

More Information: https://www.futuresamr.co.uk/

Transdisciplinary Antimicrobial Resistance Genomics Network (TARGetAMR)

Willem van Schaik[1], Paul Flowers[2], Tess Johnson[3], Stephanie Johnson[3], Nicole Wheeler[1], Kevin Dunn[1], Rivie Mayele-Tamina[1], Kate Baker[4]

[1]University of Birmingham, Birmingham, United Kingdom. [2]University of Strathclyde, Glasgow, United Kingdom. [3]University of Oxford, Oxford, United Kingdom. [4]University of Cambridge, Cambridge, United Kingdom

Abstract

The implementation of high-throughput DNA sequencing technologies ('genomics') has revolutionised our understanding of the diversity and evolution of pathogens. The TARGetAMR network (www.targetamr.org.uk) aims to bring together transdisciplinary expertise to realise the full potential of genomics and deliver~~ing~~ a step-change in the surveillance and diagnostics for antimicrobial resistance (AMR) across infection prevention and control, public health, and for informing animal husbandry and biosecurity. AMR genomics has the potential to be applied in a wide variety of settings, to improve human, animal and environmental health. We will achieve this goal by developing innovative transdisciplinary solutions to the implementation of AMR genomics, by facilitating dialogue that challenges the current perceived goals and limitations of AMR genomics, progressing innovation in research alongside considerations of social and ethical implications.

TARGetAMR is providing a platform for UK researchers from institutions across the country, and those in policy and industry, to tackle the global challenge of AMR through genomics. We currently have more than 200 members in our network, and on our community hub, and lead a series of webinars, meetings, and catalyst grant opportunities to share progress and insights in the field of AMR genomics. The network will break down disciplinary silos that inhibit progress and translation in the AMR genomics field and focus on identifying a series of transdisciplinary research questions that will deliver on the potential of AMR genomics to improve human and animal health.

The AMAST Network – A powerful and informed new voice in the One Health approach to antimicrobial resistance

K. Marie McIntyre ORCID iD[1], Lucy Brunton ORCID iD[2], Mahmoud Eltholth ORCID iD[3], Andrew Desbois ORCID iD[4], Lisa Marchioretto ORCID iD[5], Matthew Gilmour ORCID iD[5]

[1]Newcastle University, Newcastle, United Kingdom. [2]Royal Veterinary College, London, United Kingdom. [3]Royal Holloway University of London, London, United Kingdom. [4]University of Stirling, Stirling, United Kingdom. [5]Quadram Institute, Norwich, United Kingdom

Abstract

Antimicrobial resistance (AMR) of microbes is a growing threat that affects everyone, leading to treatments that should work against infectious diseases no longer working. Within the agrifood sector, when considering crops, farm animals and foods, this is a challenge that can be solved by continued coordinated action between those with roles in the use of antimicrobials alongside researchers, who together, can help answer how risks of resistance arise and can be mitigated. Proving what is possible, the UK agrifood sector have reduced usage and selectively use antimicrobials, and there are further opportunities to develop. The AMAST (Antimicrobial Resistance in Agrifood Systems Transdisciplinary) Network is a significant new collaboration in the UK's fight against AMR, bringing together key agrifood stakeholders to establish a comprehensive research framework to address AMR from farm-to-fork perspectives. With this mandate, and importantly, with the cross-sectoral collaboration within the network, AMAST will inject a powerful and informed new voice for agrifood stakeholders into the discussion of AMR in the UK. AMAST is currently establishing the foundation for a transdisciplinary approach to tackling AMR challenges in the agrifood sector using a systems-based approach. For example, AMAST is organising workshops with animal producers and veterinarians to understand if continued reductions in antimicrobial use are possible, while still prioritising health and welfare of farm animals. The AMAST strategic roadmap emphasizes the importance of collaborative action, and such activities with our partners will enable us to create authentic, industry-focused solutions to AMR, grounded in food production behaviour realities and economic decision-making.

ARREST-AMR: Advancing One Health Diagnostics to Combat Antimicrobial Resistance

Seshasailam Venkateswaran

Queen Mary University, London, United Kingdom

Abstract

Antimicrobial resistance (AMR) is a global crisis, demanding urgent, coordinated actions. Diagnostics, alongside therapies and vaccines, form the three vital lines of defense against AMR. However, nearly a decade after the O'Neill report's call for diagnostic-guided treatments by 2020, fit-for-purpose diagnostics remain elusive.

A One Health approach is critical to tackling AMR across interconnected domains of humans, animals, plants, and the environment. With 73% of global antimicrobial use in food animals and over 75% of emerging human pathogens originating in animals, diagnostics are essential for timely detection in livestock. Similarly, resistance to fungicides jeopardizes plant health and raises concerns about its role in antifungal resistance in human pathogens. Environmental drivers like pollutants and antimicrobials further exacerbate AMR, underscoring the need for targeted diagnostics to monitor and mitigate risks.

The clinical impact of AMR is stark - bacterial pathogens alone account for 5 million annual deaths, with fungal pathogens posing a growing threat. ARREST-AMR addresses these challenges through a One Health focus, driving transdisciplinary collaboration to create impactful solutions.

Our approach focuses on five objectives:

1. **Identify Needs**: Engage stakeholders across sectors to pinpoint priority areas for diagnostics.
2. **Innovate**: Foster research to address identified needs, leveraging diverse expertise.
3. **Evaluate**: Develop standardised methods for assessing performance and utility.
4. **Implement**: Tackle translational, regulatory, and adoption barriers.
5. **Cross-Pollinate**: Share best practices and insights within and beyond sectors.

With UK's scientific excellence, ARREST-AMR aims for transformative progress in AMR diagnostics to safeguard global health.

IMPACT AMR: a transdisciplinary network.

Clare Chandler[1], Julie Robotham[2], Laura Richards[3], Emiliano Ariel Videla Rodríguez[1], Benjamin Parcell[4], Steven Montgomery-Laird[5], Dominic Moran[6]

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Abstract

The IMPACT AMR is a transdisciplinary network, funded by the UKRI, oriented towards prioritising interventions to minimise the burden of antimicrobial resistance. The IMPACT AMR Network takes as its starting point the policy question, "Where should limited resources be allocated to have the greatest impact on AMR?". Therefore, the network exists to support shared working across disciplines, sectors, policy, and practice, as well as those affected by AMR and its interventions. The overall aims of the network are to i) define, from a multi-stakeholder perspective, the impact(s) of AMR-focused interventions, ii) establish a transdisciplinary framework for prioritizing among AMR interventions across the One Health spectrum, and iii) catalyse new transdisciplinary research towards developing and evaluating interventions to maximise AMR intervention impact based on identified evidence gaps. The IMPACT AMR Network is composed of four workstreams intending to combine efforts to produce a framework, enabling parsimonious approach to addressing the drivers of AMR, identifying the 'best buy' interventions to invest in as well as those that could be de-implemented, in addition to gaps where new interventions are needed. The network will connect and expand the community of researchers and stakeholders guided by the principles of a shared focus on the goal of improving prioritisation of AMR interventions, diversity of its constituents, and equality within collaboration. The IMPACT AMR Network will hold engagement activities over its four years including online meetings, face to face workshops, and a conference, together with communications including newsletters, social media, and a website.

Surveillance of Antimicrobial Resistance in clinical infections from companion animals (VetCLIN AMR)

Dorina Timofte ORCID iD, Alan Radford, Gina Pinchbeck, Ashley Ward, April Lawson, Flavia Zendri, Peter-John Noble, Shirley Bonner

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Abstract

Antimicrobial resistance (AMR) is one of the most concerning heath issues for both animals and humans. Monitoring the potential for AMR inter-species transmission requires the establishment of robust surveillance systems aligned with One Health principles. Whilst AMR surveillance in human clinical infections and in food-producing animals is well coordinated at European level (1-2), there are no established European systems to monitor AMR in clinical bacterial isolates from animals (3). Recently, to address this issue, the EU Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI), proposed to establish the European Antimicrobial Resistance Surveillance network in Veterinary medicine (EARS-Vet) for monitoring AMR in diseased animals and to strengthen the One Health AMR surveillance approach in Europe (4). Another important issue is the lack of a European or national consensus for methodologies used in veterinary laboratories for performing and interpreting bacterial cultures from companion animal clinical specimens or for antimicrobial susceptibility testing (AST), hampering the comparability of these data (5).

In the UK, the VetClin AMR project [funded by Veterinary medicine Directorate under the National Biosurveillance Network (NBN)] is filling the gap on surveillance of AMR in clinical infections from companion animals by collaborating with the private veterinary laboratories (PVLs) to generate both passive and active AMR surveillance data. Furthermore, the project uses methodologies for bacterial culture, AST and whole genome sequencing – aligned with EARS-VET, and is working with PVLs towards harmonisation of laboratory methodologies, so the data generated in the UK is comparable between UK labs and with other European countries.

Evaluation of Climate Change Impacts on AMR Using a Planetary Health Framework (CLIMAR)

William Gaze ORCID iD[1], Jennifer Cole ORCID iD[2], Paul Kadetz ORCID iD[3], Rebecca King[4], Lea Berrang Ford ORCID iD[5]

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Abstract

Climate change and antimicrobial resistance (AMR) are complex challenges that pose significant threats to society. The triple planetary crisis of climate change, pollution and impacts on biodiversity, highlighted by the UN, are likely to impact AMR emergence and transmission. It is essential to account for the social, cultural and physical environments of AMR, including the impacts of climate change. Increasing temperatures and changing patterns of rainfall will affect AMR evolution and transmission, patterns of human migration, and will change food production, land use and freshwater use. Conversely, antimicrobials may impact microbial geochemical cycling, such as nitrogen cycling in soils and methane production in ruminant microbiomes. These interactions raise the intriguing possibility that a bidirectional relationship exists between climate change and AMR.

The aim of the CLIMAR network is to explore the intersection of climate change, planetary health and antimicrobial resistance (AMR) and the impact of humans on these complex interactions. This will be supported through collaboration between researchers, business leaders and policy makers to inform mitigation and adaptation strategies, in addition to other best practices. The transdisciplinarity essential to this work is supported by the structure of the Planetary Boundaries and the themes and concepts informed by the Planetary Health Educational Pillars. Considering AMR drivers at a “planetary scale” facilitates collaboration across disciplines and sectors that can build the evidence base for interventions that control the development of AMR and its impacts on health and society.

AMR education: One Health approach

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Abstract

Antimicrobial resistance (AMR) is a significant public health issue, and it is critical that AMR and related topics are covered adequately in health and allied health programs. Education delivered to students enrolled in programs such as medicine, dentistry, pharmacy and veterinary science is increasingly being taught within a One Health framework. This highlights the need for high-quality AMR teaching resources with a One Health focus, to support educators teaching into various health and allied health disciplines.

While some educators may be developing AMR resources for use in their own teaching, having readily available teaching materials and resources on AMR, hosted on the Microbiology Society website, would be invaluable for members.

This project will involve a collaboration of microbiology educators from various institutions to review existing resources and develop new resources, aimed at promoting multidisciplinary active learning on AMR topics. In addition to health and allied health professionals, AMR is relevant to non-health-specific disciplines including agriculture and environmental science. This interdisciplinary context will be carefully integrated when designing resources. The developed resources will be externally benchmarked where possible and formally evaluated following implementation in the classroom; this evaluation will be published in *Access Microbiology.*

Beyond enhancing microbiology education, this project is also expected to contribute towards advancing AMR research more broadly in the future.

The Role of Microbiomes in Humans, Animals, and Ecosystems

Invited: Exploring dynamics of early life microbiomes

Lindsay Hall

University of Birmingham, Birmingham, United Kingdom. Quadram Institute Biosciences, Norwich, United Kingdom

Abstract

The early life developmental window is a critical period during which the microbiota exerts significant influence on host health. This talk will explore the intricate dynamics of microbial communities, with a particular focus on *Bifidobacterium* species and their genomic diversity and interplay with other key bacterial species and strains. Our research delves into the mechanisms by which these microbial communities contribute to the development of vital host pathways, including immune regulation and neurodevelopment during pregnancy and infancy. I will also present findings from our work on how external factors such as diet and antibiotic exposure, impact the initial microbiota colonisation and the downstream health outcomes for both mothers and infants. Furthermore, I will discuss how targeted strategies aimed at restoring microbial ecosystem dynamics - including the use of probiotics, prebiotics, and specific microbial components - may be used to improve short- and longer-term health.

Invited: Translating microbiome research into clinical practice.

Jack Gilbert ORCID iD

University of California San Diego, La Jolla, USA

Abstract

The human microbiome is a high dimensional and dynamic part of our physiology that plays a key role in managing health and individualized responses to diet and medicine. The immune system controls our interaction with the microbial world, and the microbial communities in our bodies are central to modulating the immune response. Changes in the human microbiome and their metabolism have substantial influence on atopy, neurological disorders, metabolic disorders, and a range of complex conditions and disease states. Diet is incredibly important in shaping human health and the microbiome, altering both composition and metabolic activity, resulting in changes in immune, endocrine, and neurological systems. Microbiome-Wide Association Studies (MWAS) combined with novel quantitative multi-omic approaches are enabling us to use AI techniques to determine personalized responses to nutrition that drive diseases states and treatment efficacy. Through these innovations we are finally realizing the paradigm of precision medicine for facilitating patient care.

Invited: Understanding the rumen microbiome to enhance methane mitigation strategies for ruminants

Sinead Waters ORCID iD

University of Galway, Galway, Ireland

Abstract

The rumen microbial community provides ruminants with a unique ability to convert human indigestible plant matter, into high quality proteins. However, CH_4 produced in the rumen is both a potent greenhouse gas and a metabolizable energy loss for ruminants. As the rumen microbiome constitutes 15-40% of the inter-animal variation in enteric CH_4 emissions, understanding the microbiological mechanisms underpinning ruminal methanogenesis is crucial for developing CH_4 mitigation strategies. Differences in the rumen microbiome composition were observed in cattle with contrasting residual methane emission and CH_4 yield. Low emitters had an increased abundance of bacteria producing lactic acid, succinate and propionate, while in high emitters, H_2 producing microbes were increased. Indeed, the relative abundance of three ruminal bacteria and the Methanobrevibacter SGMT clade, accounted for 20% of the variation in CH_4 emissions. However, the demonstration of ruminotypes associated with high or low CH_4 emissions suggests that interactions within complex microbial consortia are a major source of variation in CH_4 emissions. Consequently, microbiome-assisted genomic approaches are being developed to select low CH_4 emitting cattle, with breeding values for enteric CH_4 being included as part of national breeding programmes. Understanding the rumen microbiome has also aided the development of anti-methanogenic feed additives. Current research aims to provide alternative hydrogen sinks and to stimulate activity of commensal microbes or the direct supplementation of direct-fed microbials to capture this lost energy. Furthering our knowledge of the rumen microbiome aids in the development of methane mitigation strategies for ruminant livestock.

Invited: Virome-bacteriome interactions in the gut microbiome; are bacteriophage and bacteria fighting or dancing?

Colin Hill ORCID iD

University College Cork, Cork, Ireland

Abstract

Bacteriophage may be the most abundant biological entities on the planet, but obviously require sensitive bacterial host for their replication. The interaction of an individual bacterial cell encountering a bacteriophage is often portrayed as a fight to the death (or to a standstill in the case of lysogenic phage). Either the phage kills the cell, or a resistant cell prevents the phage from replicating successfully. Both outcomes are catastrophic for the loser. However, at the level of bacterial and phage communities the interaction can be portrayed as a dance between partners with mutual benefits. Phage benefit from the presence of viable hosts to allow their continued reproduction and survival, but bacteria can also benefit from the presence of predators driving heterogeneity within otherwise clonal populations. I will use specific examples involving the most abundant pahge in the human gut, the crAssvirales, and their bacterial hosts to illustrate the mutual benefits that can accrue from this ongoing relationship that has persisted and thrived for billions of years.

Longitudinal analysis of the gut microbiome in adolescent patients with anorexia nervosa: microbiome-related factors associated with clinical outcome

Arunabh Sharma ORCID iD[1], Nadia Andrea Andreani[2,3], Brigitte Dahmen[4], Hannah E. Specht[4], Nina Mannig[4], Vanessa Ruan[4], Lara Keller[4], John F. Baines[2,3], Beate Herpertz-Dahlmann[4], Astrid Dempfle[1], Jochen Seitz[4]

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Abstract

There is mounting evidence regarding the role of gut microbiota in anorexia nervosa (AN). Previous studies have reported that patients with AN show dysbiosis compared to healthy controls (HCs); however, the underlying mechanisms are unclear, and data on influencing factors and longitudinal course of microbiome changes are scarce. Here, we present longitudinal data of 57 adolescent inpatients diagnosed with AN at up to nine time-points (including a 1-year follow-up) and compare these to up to six time-points in 34 HCs. 16S rRNA gene sequencing was used to investigate the microbiome composition of fecal samples, and data on food intake, weight change, hormonal recovery (leptin levels), and clinical outcomes were recorded. Differences in microbiome composition compared to HCs were greatest during acute starvation and in the low-weight group, while diminishing with weight gain and weight recovery at the 1-year follow-up. Illness duration and prior weight loss were strongly associated with microbiome composition at hospital admission, whereas microbial changes during treatment were associated with kilocalories consumed, weight gain, and hormonal recovery. The microbiome at admission was prognostic for hospital readmission, and a higher abundance of *Sutterella* was associated with a higher body weight at the 1-year follow-up. Identifying these clinically important factors further underlines the potential relevance of gut microbial changes and may help elucidate the underlying pathophysiology of gut-brain interactions in AN. The characterization of prognostically relevant taxa could be useful to stratify patients at admission and to potentially identify candidate taxa for future supplementation studies aimed at improving AN treatment.

Bacterial microcompartments and energy metabolism drive gut colonization by Bilophila wadsworthia

Lizbeth Sayavedra ORCID iD

Quadram Institute Bioscience, Norwich, United Kingdom

Abstract

High-fat diets alter the gut microbiota composition and stimulate proliferation of the sulfidogenic bacterium *Bilophila wadsworthia*. Proliferation of *B. wadsworthia* is linked to gut inflammation, dysfunction of the intestinal barrier and bile acid metabolism but the genetic basis for its colonization of the gut remains unknown.

Here, we identified genes that facilitate germ-free male mice gut colonization by *B. wadsworthia* under a high-fat diet, with or without a simplified humanized microbial consortium (SIHUMI). Using genome-wide transposon mutagenesis, metatranscriptomics and untargeted metabolomics, we identified 34 genes essential for gut colonization. These included two gene clusters related to a bacterial microcompartment (BMC), and a NADH dehydrogenase (*hdrABC-flxABCD*) important for energy metabolism in anaerobes. BMCs allow *B. wadsworthia* to metabolise the organosulfonate compounds taurine and isethionate -abundant in the mammalian gut- releasing H_2S, acetate and possibly ethanol. Although the H_2S concentration and *B. wadsworthia* abundance were at their highest in the absence of the SIHUMI, detrimental impacts on the host were exacerbated in the presence of the SIHUMI, based on gut permeability, and increased infiltration of macrophages in the liver. Our findings suggest that in the presence of other microbes, *B. wadsworthia* may switch to using alternative energy sources like lactate or formate, exacerbating its harmful effects on the host. Thus, microbial community interactions, rather than H_2S alone, play a critical role in modulating *B. wadsworthia*'s impact on host health.

Global Air Microbiome Encyclopedia: AI-Enhanced Metagenomics for Species-Level Analysis of Airborne Microbial Diversity

Elena S. Gusareva ORCID iD[1,2], Lennard Wittekindt[1], Kutmutia Shruti Ketan[1], Vineeth Kodengil Vettath[1], Lakshmi Chandrasekaran[1], Sam Spence[1], Justine J.A. Dacanay[1], Rebecca J. Case ORCID iD[1,3], Stephan C. Schuster ORCID iD[1,3]

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Abstract

Artificial intelligence (AI) holds vast potential in microbiology, especially for advancing our understanding of airborne microbial communities. In this study, we developed the Global Air Microbiome Encyclopedia (GAME), an AI-powered web-based system for metagenome annotation and data refinement, to analyze the distribution and diversity of airborne microorganisms worldwide. GAME provides insights into the environmental and climatic influences on airborne microbial dynamics, with significant applications in planetary ecosystem studies and public health policy.

Using metagenomic sequencing, we analyzed 1,175 air samples from seven continents, comprising 1.6 terabases of sequenced DNA, to identify airborne bacteria and fungi. We identified 20% (20,734) of bacterial and 5% (1,525) of fungal taxa in a non-redundant sequence database, with up to 50% of microbial taxa confirmed to species-level. Our biogeographic analyses revealed distinct distribution patterns for microbial taxa: bacteria were mostly locally dispersed, dominated by soil-associated species, while fungi showed broader, often global, dispersal, with plant-associated species dominating. Antarctic samples mostly shared microbial diversity with nonpolar regions, suggesting polar areas act as sinks for global bioaerosols.

Our GAME system provides access to the extensive dataset on airborne microbial diversity and global distribution, enabling a deeper understanding of bioaerosols and their potential impacts on ecosystems and health.

Next Generation Probiotics – 'Omics-based Identification of Candidate Bacteria in the Thoroughbred Foal Microbiota' – The Bioinformatic Phase

Jack Whitehouse, Carla Moller-Levet, Arnoud van Vliet, Joy Leng, Roberto La Ragione, Christopher Proudman

University of Surrey, Guildford, United Kingdom

Abstract

Early-life gut microbial community structure can influence the development of microbe-host and microbe-microbe interactions. Therefore, the gut microbiota may contain bacterial species that have the potential to convey health benefits in the form of next-generation probiotics. However, there is limited evidence on the efficacy of many probiotics currently used in horses. In this study, the gut microbiota of 20 Thoroughbred foals obtained from the Alborada Well Foal Study were profiled using faecal samples at: 2, 8, 14, 28, 60, 90, 180, 272, 365-days old. Samples were analysed using shotgun metagenomic sequencing, processed with SqueezeMeta, and modelled against health data collected for orthopaedic, soft tissue, respiratory and gastrointestinal health events. A non-metric multidimensional scaling model indicated that three species were responsible for driving changes in the gut microbiota structure in the first year of life, these were *Escherichia coli*, *Streptococcus agalactiae*, and *Acinetobacter johnsonii*. Based on these data, a multivariable Cox-mixed-effects model was constructed for each sampling time-point to assess the relationship between relative species abundance and age of first diagnosis of a health event. This analysis indicated that 327-species were significantly associated with reduced risk of future health events ($p<0.05$). The 327-species were cross-referenced with selection criteria (e.g., size ofhazard ratio) to determine which species offer the highest degree of health benefits, before being cultured in line with EFSA qualified presumption of safety for microbe assessment (i.e., genotypic and phenotypic analysis). These findings provide an evidence-base of potential next-generation probiotics with the potential to deliver health benefits for Thoroughbred foals.

Monitoring The Salivary Microbiome To Elucidate Their Functional Response To Oral Actives

Chandra Lekha Ramalingam Veena ORCID iD[1], Jagroop Pandhal[1], David Bradshaw[2], Graham Stafford[1]

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Abstract

The oral microbiome is a diverse community of microorganisms, including commensals, symbionts, and pathogens. While previous studies have classified microbes in healthy and diseased states, the functional changes in response to low doses of antimicrobials are less understood. This study focuses on how sub-lethal doses of Chlorhexidine (CHX), Stannous fluoride (SnF2), and a novel compound, 2-isopropyl-5-methylphenol (IPMP), impact the oral microbiome. We also examine how individual bacterial species develop tolerance to CHX and assess the biofilm's surface changes using imaging techniques. To investigate, biofilms derived from healthy human saliva were subjected to 16S rRNA sequencing to assess changes in microbial composition, while proteomic analysis using liquid chromatography-mass spectrometry identified protein changes in treated biofilms. CHX tolerance was monitored by culturing bacterial species on CHX-supplemented agar, and disk diffusion assays were used to assess antibiotic resistance in strains showing CHX tolerance. Microscopy techniques, including LIVE/DEAD staining, scanning electron microscopy (SEM), and Eosin-Y fluorescence, provided insights into the biofilm's structural alterations. Salivary biofilms subjected to 16S rRNA sequencing revealed that bacterial biofilms treated with CHX, SnF2, and IPMP exhibited altered compositions of specific oral commensals. Metaproteomic analysis revealed differences in the proteome of treated biofilms. Passaging indicated tolerance to CHX and changes in the minimum inhibitory concentration (MIC). Decreased sensitivity to common antibiotics was noted after passaging. Biofilm imaging revealed altered composition, viability, and changes to the biofilm matrix. This study offers new methods to explore how routine oral antimicrobials impact the dynamic oral microbiome and microbial community interactions.

Viral and microbial contributions preceding disease onset in preterm infants: a cross NICU study in the UK

Rui Santos ORCID iD[1], Andrew Nelson[1], Gregory Young[2], Mohammad Tariq[1], Wen Yew[1], Jasmine Jessop[1], Nicholas Embleton[3], Christopher Stewart[2], Janet Berrington[2,3], Darren Smith[1]

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Abstract

Very low birthweight preterm infants with less than 32 weeks of gestational age are more susceptible to develop diseases such as necrotising-enterocolitis (NEC) and development of late-onset sepsis (LOS). In the UK, 1 in 12 babies are born prematurely. In addition, 1 in 20 of these preterm infants (< 32 weeks of gestational age) are at risk of developing NEC, and an associated higher mortality rate of >20%. Development of a stable gut bacterial microbiome plays an important role in early child health, however the role of the gut virome in preterm infant's health and disease early in life remains underexplored. We characterised the gut virome (NovaSeq 6000, Illumina) and microbiome (full-length 16S rRNA PacBio Kinnex) of 39 preterm infants that developed NEC and/or LOS, and 71 healthy preterm infants matched for gestational age and neonatal intensive care unit (NICU). This study importantly does not only focus on dsDNA bacteriophages, but also ssDNA and RNA bacteriophages from preterm infants admitted to 13 different NICU sites across England. A smaller subset (74 stool samples of 395) involving 7 preterm infants that developed disease, and their matched controls was used to target ssDNA and RNA bacteriophages. The data and expansion of dsDNA phage diversity illustrates that the viral compartment is a better indicator of the development of NEC.

Balance of bile acid conjugation and hydrolysis reveals potential for gut microbiome to shape liver physiology and fat absorption

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Abstract

The human gut microbiome is responsible for many forms of bile acid (BA) modifications, including deconjugation and reconjugation via microbial bile salt hydrolase (BSH). The newly discovered reconjugation is an acyl-transfer of an amino acid to produce diverse microbially conjugated bile acids (MCBAs). Because BAs are responsible for essential functions in the gut and liver including fat absorption, metabolic receptor signaling, and shaping microbiome structure, microbial BSH activity is the molecular fulcrum balancing these vital functions. This study explored how BSH's hydrolytic and acyl-transfer activity varies across gut microbes and alters GI physiology in cell culture and a mouse model. Anaerobic culturing of 17 gut bacteria exposed to host-conjugated taurocholic acid (TCA), glycocholic acid (GCA), or MCBAs (including Ala, Asp, Glu, Leu, Phe, Ser, Thr, and Tyr conjugates) revealed varied hydrolytic and acyl-transfer profiles between microbes. Although TCA and GCA were readily hydrolyzed, MCBAs such as ThrCA and GluCA, were more resistant and overall BSH hydrolysis was low, despite an annotated BSH gene in the genome. We explored how different conjugated BAs alter cellular receptor signaling and toxicity. PheDCA was particularly cytotoxic with 50% of cells dying at 100 µM compared to TDCA, which reduced viability only 14%. In a TGR5 signaling assay, LeuCA halved TGR5 agonism at 30 µM compared to TCA. Furthermore, feeding mice MCBAs showed that they can act systemically via enterohepatic circulation. These experiments show that BSH acyl-transfer activity may tip the balance of BA functions in the human gut with implications for many gastrointestinal diseases.

Fusobacterium is toxic for head and neck squamous cell carcinoma and its presence may determine a better prognosis

Anjali Chander[1,2], Jacopo Iacovacci[3], Aize Pellon Rodriguez[1], Victoria Butterworth[2], Radhika Kataria[1], Anita Grigoriadis[1], John Maher[1], Cynthia Sears[4], Gilad Bachrach[5], Teresa Guerrero Urbano[1,2], Mary Lei[1,2], Imran Petkar[1,2], Anthony Kong[1,2], Tony Ng[1], Ester Orlandi[3], Nicola Alessandro Iacovelli[3], Loris De Cecco[3], Mara Serena Serafini[3], David Moyes[1], Tiziana Rancati[3], Miguel Reis Ferreira[1,2]

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Abstract

Head and neck squamous cell carcinoma (HNSCC) typically presents at advanced stages, with poor survival outcomes following standard therapies. Emerging evidence indicates that the oral microbiome may influence cancer therapy efficacy. We analysed microbiome data from two independent patient cohorts to explore associations between oral bacteria and HNSCC treatment efficacy and validated these findings using in vitro co-culture models.

Within both cohorts, it was observed that patients with a higher relative abundance of tumoral *Fusobacterium nucleatum* and salivary *Fusobacterium*, had improved survival outcomes. These findings were supported by in vitro 2D co-culture data within which *Fusobacterium nucleatum* actively reduced oral cavity squamous cell carcinoma (OSCC) cell viability; this effect was replicated across multiple OSCC cell lines (TR146, HN5 & HSC3) as well as a dysplastic oral keratinocyte cell line. This is in addition to with two wild type *F. nucleatum* strains (ATCC 23726 & ATCC 25586). This effect was specific to *F. nucleatum* as OSCC cell line infection with *Prevotella oralis* (NCTC 11459), which is also a Gram-negative commensal oral anaerobe, did not cause significant OSCC cell kill. Additionally, co-culture irradiation experiments revealed that *F. nucleatum* enhanced the cytotoxic effects of radiotherapy, particularly when introduced post-irradiation, thus suggesting a potential radiosensitising role of *F. nucleatum*.

These findings support *Fusobacterium* as a promising prognostic biomarker in HNSCC. Ongoing research is currently being undertaken to elucidate mechanisms and further investigate its role in radiotherapy-mediated HNSCC cytotoxicity.

The fungus among us: confronting antimicrobial resistance in fungi

Invited: Searching for the source: how environmental fungi become clinically resistant

Amelia Barber

Friedrich Schiller University, Jena, Germany

Abstract

Many human fungal pathogens reside primarily in the environment and there is an overlap in the mechanism of action in compounds like the azoles that are both important plant fungicides and frontline medical antifungals, suggesting that agricultural azole use may be driving clinical resistance. To examine this, we performed systematic soil sampling on cereal crops and apple orchards in Germany before and after azole fungicide application for three years and quantified their impact on *Aspergillus fumigatus*. This organism is an environmental saprobe and WHO priority pathogen responsible for an estimated 300,000 deaths annually. While azole fungicide exposure was associated with a reduction in the abundance of *A. fumigatus*, not all fields and years displayed this trend, indicating that other factors also influence its abundance in soil. Isolates collected after the growing season and azole exposure show a mild decrease in susceptibility to medical and agricultural azoles. However, MICs largely stay below the breakpoint for clinical resistance and the overall resistance frequency among agricultural isolates was low, with only 1-3% of isolates collected over the three years being clinically resistant. Whole-genome sequencing indicated that fungicide application did not significantly affect the population structure and genetic diversity of *A. fumigatus* in fields. We conclude that azole use on crops is not significantly driving resistance in *A. fumigatus*, but there may be additional contexts where their usage leads to clinical resistance, which will be critical to identify and mitigate, as there are preciously few approved antifungals available for treating fungal infections in humans.

Invited: Rising Heat, Rising Threat: How Climate Change Fuels Fungal Pathogens

Norman van Rhijn

University of Manchester, Manchester, United Kingdom

Abstract

Climate change is driving significant changes in the spread and evolution of fungal pathogens, posing new challenges to human health. Rising temperatures, shifting boundaries, and increased fungicide use affect fungal pathogens, leading to new risks and wider spread. Saphrotrophic fungi like *Aspergillus fumigatus* and *Aspergillus flavus* are adapting to these environmental pressures, showing different geographical distribution in response to a changing climate. In addition, rapidly changing environments can give rise to novel pathogens as is exemplified by *Candida auris*, a multidrug-resistant fungus recently recognized as a major health threat. Climate change may even be accelerating genetic mutations in some fungal pathogens, allowing them to develop resistance to existing treatments faster than before.

In this talk I will highlight key findings from recent research on how climate change affects fungal diseases and examine predictive models that forecast changes in disease patterns. By combining data from ecology, epidemiology, and genetics, we aim to provide a clearer picture of how these climate-driven shifts in fungal pathogens may impact disease.

Invited: Azole Resistance in the Apple Scab Pathogen Controlled by Dose-Dependent Genetic Mechanisms

Thomas Heaven[1], Andrew Armitage ORCID iD[2], Xiangming Xu ORCID iD[3], Matthew Goddard ORCID iD[4], Helen Cockerton ORCID iD[5]

[1]John Innes Centre, Norwich, United Kingdom. [2]Natural Resources Institute, Kent, United Kingdom. [3]NIAB, Kent, United Kingdom. [4]University of Lincoln, Lincoln, United Kingdom. [5]University of Kent, Canterbury, United Kingdom

Abstract

Antimicrobial resistant fungi represent a major challenge for the agricultural industry and pose a significant threat to food production. Chemical fungicides are relied upon to protect crop plants from plant diseases.

Apple orchards are routinely sprayed with a series of chemical fungicides in order to protect them against disease and in turn, boost yields and produce high quality food. One of the most economically damaging pathogens in apple production is *Venturia inaequalis* which causes apple scab lesions on both leaves and fruits. Recurrent application of fungicides has provided the selection pressure required for the emergence of azole resistance across multiple apple scab pathogen populations. Typically, resistance incidences are caused by a point mutation in the CYP51 gene, however, these target site mutations do not explain all cases of resistance. Here we study a population of *V. inaequalis* containing resistance to tebuconazole. Through genotyping-by-sequencing a population of scab fungi segregating for polygenic resistance, we were able to map two Quantitative Trait Loci contributing in varying degrees to resistance at different doses of fungicide application. We observe non-synonymous mutations in several transporter genes in the QTL region leading us to hypothesise that fungicide resistance is caused, in part, by chemical efflux. These findings support the development of comprehensive pathogen diagnostics to inform fungicide resistant management strategies.

Invited: Exploring the activity of hydrogen sulphide against dermatophytes

Albert Bolhuis ORCID iD

University of Bath, Bath, United Kingdom

Abstract

Nail infections are very common but often challenging to treat. They are most frequently caused by a group of fungi called dermatophytes, but also other fungi and bacteria can cause these infections. Oral treatments can be effective but may cause adverse effects and drug interactions, while topical treatments are often ineffective and can take over a year due to poor penetration of antifungals into the nail plate. Moreover, antifungal resistance is on the increase, and it is therefore important to develop alternative treatment strategies.

Small molecules, such as hydrogen sulphide (H_2S), are predicted to penetrate readily into the nail plate, and previous studies have shown that H_2S has antifungal properties against fungal plant pathogens. The aim of this study was therefore to test whether H_2S has effective antimicrobial activity against microbes that cause nail infections.

We found that H_2S has a strong antimicrobial activity against dermatophytic and non-dermatophytic fungi and some bacteria. Interestingly, the most active form appeared to be H_2S, not the anion HS^-, which was likely due to its faster uptake into cells. In the most common causative agent of fungal nail infections, *Trichophyton rubrum*, we demonstrated that H_2S has multiple intracellular targets, and transcriptomic studies in revealed several genes that were up- or downregulated in response to H_2S, indicating a stress response. Overall, hydrogen sulphide shows promise as a potential treatment against microbes that can cause nail infections due to its strong antimicrobial activity.

Invited: Candida albicans Utilises Methaemoglobin to Build Ultra-Drug Resistant Polymicrobial Biofilms

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Abstract

The growth of drug resistant poly-microbial biofilms represents a major clinical problem that underpins recurrent infection and failed therapy. Here we show how the presence of Methaemoglobin, (MetHb), which forms when the iron component in haemoglobin is oxidised from the ferrous (Fe2+) to the ferric (Fe3+), state is bound by *C. albcians* into dense biofilms structures that show remarkable drug resistant properties. Our analysis suggests that *C. albicans* is able to lyse red blood cells to liberate MetHb as as a structural component for biofilm establishment, as opposed to using it as a source of nutrition. The *C. albicans*/MetHb aggregate biofilms that form show increased virulence most likely linked to accelerated hyphal extension. Furthermore, we show that the presence of *C. albicans/*MetHb increases the rate of *S. aureus* and *P. aeruginosa* incorporation in biofilms. As MetHb levels are increased within a number of disease pathologies, including sepsis, *C. albicans*/MetHb aggregate biofilms may represent an as yet unexplored structure that supports metastatic infection and poor patient outcome.

Universal PCR assays to detect *Candida* FKS and ERG11 mutations

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Abstract

The emergence of antifungal resistance in *Candida* species presents significant challenges in managing infections. Mutations in the FKS genes are known to confer resistance to echinocandins, while ERG11 mutations are associated with resistance to azoles. This study aimed to develop rapid molecular assays for detecting FKS and ERG11 mutations in four clinically significant *Candida* species: *Candida albicans*, *C. glabrata*, *C. tropicalis*, and *C. parapsilosis*.

Two universal polymerase chain reaction assays were developed: one for detecting FKS mutations and another for ERG11 mutations. Also, antifungal susceptibility testing was performed against echinocandins (micafungin and anidulafungin) and azoles (fluconazole and voriconazole) in accordance with the Clinical and Laboratory Standards Institute guidelines (M27-A3 and M27M44S).

In this study, we tested the developed assays with six American Type Culture Collection type strains, six blood culture isolates, and eight laboratory-derived strains exhibiting resistance to echinocandins or azoles. In total, five FKS mutations and two ERG11 mutations were identified, and the mutations were shown to be correlated with elevated minimum inhibitory concentrations for the antifungal agents tested.

The two assays have a test turnaround time of approximately six hours for amplification and sequencing of the target regions, allowing mutation detection results to be readily available on the same day blood cultures become positive.

In conclusion, the developed universal PCR assays for detecting FKS and ERG11 mutations are potential rapid diagnostics tools for antifungal resistance. By enabling timely detection of the mutations, these assays have great potential to optimize antifungal therapy and improve patient outcomes.

Prosopis juliflora-Derived Copper Nanoparticles Combating Against *Candida albicans* Infections

Syeda Maryam Hussain[1], Muhammad Zishan Ahmad[1], Zaib Ur Rehman[1], Muhammad Ali Shah[1], Murtaz Ul Hassan[1], Muhammad Shoaib[1], Muhammad Kamran[1], Saif Ur Rehman[1], Aayesha Riaz[1], Ali Ahmad[2], Zahid Manzoor[3]

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Abstract

Candida albicans is a dimorphic fungus residing as a commensal organism but opportunistic pathogen causing a wide range of infections, collectively called as candidiasis. Emerging multidrug-resistant (MDR) *C. albicans* strains poses a significant challenge to human population. Here, we explore the sustainable approach to synthesize copper nanoparticles (CuNPs) utilizing *Prosopis juliflora* extract as a natural reducing and stabilizing agent. The synthesized CuNPs were characterized by utilizing different techniques, including UV-Vis spectroscopy, energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and scanning electron microscopy (SEM). These techniques confirmed the successful formation of stable and well-dispersed CuNPs with a diverse range of morphologies, including , irregular, spherical, cylindrical, truncated hexagonal, triangular, and prismatic in the range of 10-80 nm. The antimicrobial activity of the synthesized CuNPs was evaluated against *C. albicans* and it exhibited dose-dependent response. The maximum zone of the inhibition was observed at 600 ppm (25.5 ± 0.53 mm), followed by 400 ppm (21 ± 0.42 mm) and 200 ppm (17 ± 0.55 mm). These results indicates the potent antifungal properties of the CuNPs. Additionally, the antioxidant potential of the CuNPs was assessed using the DPPH radical scavenging assay. A remarkable reduction in free radical activity was observed, highlighting the antioxidant capabilities of the synthesized nanoparticles.

The green synthesis method employed in this study proposes a sustainable and eco-friendly alternative to conventional chemical methods for nanoparticle production. The biocompatible nature of *Prosopis juliflora*-mediated CuNPs suggests their potential application as a treatment of microbial infections such as *C. albicans*-induced candidiasis.

Mechanism of ammonium transport is critical in yeast filamentation

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Abstract

Nitrogen is often a limiting growth factor for microorganisms, thus growing in nitrogen limiting conditions is a challenge. To scavenge for nitrogen fungi will begin to grow hyphae or psuedohyphae which are also part of the induction of pathogenicity. The signal for this change in growth has been observed to come from the Mep2 ammonium transport membrane protein, a member of the Ammonium transport proteins (Amt), Methylamine permeases (Mep) and Rhesus proteins (Rh) superfamily found in all kingdoms of life. The overall structure of these proteins is highly conserved including specific amino acids lining their hydrophobic pore. Using *in vivo* yeast complementation, *in vitro* electrophysiology and *in silico* molecular dynamics simulations it has been found that the twin his motif, found in the centre of the pore, plays an important role in the mechanism and selectivity of Mep2. Mutations to the twin his motif switches Mep2 from a highly selective ammonium transporter to an unselective ion channel. This change has also been shown to abolish the induction of filamentous growth. This leads to the conclusion filamentous growth is not triggered by a signal cascade following ammonium translocation but rather by the specific mechanism of transport.

Diketopiperazines as scaffolds for anti-*Candida* drugs

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Abstract

Invasive fungal infections (IFIs) are a frequently overlooked driver of patient morbidity, mortality and healthcare cost. Despite the growing global concern regarding AMR, IFIs receive little attention and resources. *Candida albicans* in particular often causes IFIs, with long-term consequences in patients with compromised or poorly developed immune systems. 2,5 Diketopiperazines (DKPs), with biological activity against a range of bacterial and fungal pathogens, can act as scaffolds in anti-fungal drug design, allowing prediction of potential compound effectiveness against *C. albicans*.

This study aimed to assess DKPs, containing combinations of proline, tryptophan, tyrosine and phenylalanine to predict anti-*C.albicans* activity.

DKPs were synthesized according to Milne (1992). Anti-fungal activity was determined according to the EUCAST method for MICs in fermentative yeasts. DKPs were double diluted from glycerol stocks in RPMI-1640 from 1mg/ml. Plates were incubated in ambient air at 37°C for 24 hours and read on a microplate reader at 530nm. Response curves according to Lambert (2011) were plotted to elucidate the DKPs' mechanism of action.

Although all of the cyclic dipeptides inhibited the growth of the *C.albicans*, activity of cyclo(Tyr-Pro) at 125µg/ml, cyclo(Phe-Pro) at 250µg/ml, and cyclo(Trp-Tyr) as well as cyclo(Phe-Trp) at 125µg/ml were the most effective. From the response curves, four separate mechanisms of action were elucidated for DKPs. Those containing proline were most effective and acted via receptor-like mediated pathways.

We found that DKPs affect *C.albicans* growth with potentially four separate mechanisms of action, making them valuable models for the development of new anti-fungal drugs.

Impact of fungi on the effectiveness of antimicrobials against Pseudomonas aeruginosa in polymicrobial biofilms

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Abstract

Current in vitro biofilm models assessing the effectiveness of novel antimicrobials do not consider the impact of cohabiting microbes and CF environment on antimicrobial effectiveness often resulting in clinical failure. We aim to develop accessible polymicrobial biofilm models that are highly adaptable to CF conditions and assess the impact other bacteria and fungi on the effectiveness of antibiotics against *Pseudomonas aeruginosa*. In this study, we use a recently developed polymicrobial colony biofilm model to assess the role of *Candida albicans* and *Aspergillus fumigatus* on the antimicrobial tolerance of *P. aeruginosa* biofilms grown in tri-species biofilms with *Staphylococcus aureus*. These biofilms were treated with a range of antimicrobials and antifungals commonly used to treat CF-related infections as individual treatments and combination therapy. The effectiveness of these were determined as colony-forming units and metabolic activity enabling the calculation of minimal biofilm inhibitory concentrations. The presence of these fungi resulted in distinct biofilm morphologies and spacial microbial distribution. Within polymicrobial biofilms, the presence of *A. fumigatus* significantly reduced *P. aeruginosa* tolerance to all tested antibiotics compared to *C. albicans*. The use of antifungals highlighted significant differences between mono and polymicrobial biofilms with increased amphotericin B activity against *C. albicans* in polymicrobial leading to a parallel CFU reduction in *S. aureus* suggesting strong population dynamics not observed in polymicrobial biofilms with *A. fumigatus*. This work highlights the need to consider the polymicrobial nature, species diversity and potentially key role that fungi play in altering the effectiveness of antimicrobials on *P. aeruginosa* polymicrobial biofilms.

Cecacins, a novel group of chicken ceca microbiome derived peptides for treatment of multidrug resistant *Nakaseomyces glabrata*

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Abstract

By 2050, antimicrobial resistance (AMR) is projected to cause over 10 million deaths and result in an economic loss of £66 trillion. Fungal pathogens, especially *Candida* species, have recently emerged as an AMR threat and public health concern. This study evaluated six novel antimicrobial peptides (AMPs) Cecacin 9, 55, 56, 77, 94, and 103 identified from chicken ceca microbiomes for their effectiveness and potential synergistic effects with existing antifungal drugs against multidrug-resistant strains of *Nakaseomyces* (*Candida*) *glabrata*, a high-priority fungal pathogen with intrinsic resistance to fluconazole and cross-resistance to multiple antifungal classes.

The AMPs exhibited potent anticandidal activity with minimum inhibitory concentrations (MICs) of between 16-128 µg/mL against *N. glabrata ATCC 2001* and *DSM 11950*. Cecacins 9, 55, 77 and 103 had synergistic interactions with Amphotericin B reducing the MICs of Amphotericin by 4-fold. Cecacins 55 and 77 were synergistic with fluconazole to which *N. glabrata* is intrinsically resistant, reducing concentrations of fluconazole required for effective killing to within the susceptible range. These synergistic combinations showed potent fungicidal activity against *N. glabrata* cells compared to single activity with >4log CFU/mL reductions within 60-180 minutes of exposure and were able to significantly prevent and eradicate biofilms. Electron micrographs of *N. glabrata* treated cells showed evidence of membrane action and leakage of cell content. Cecacins also showed minimal cytotoxicity against human red blood cells and mammalian cell lines showing promise of their high therapeutic index and suitability for further development into antifungal therapies for *N. glabrata* and other *Candida* infections.

Identification and investigation of antifungal heteroresistant subpopulations in echinocandin treatment- refractory Candida auris bloodstream isolates

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Abstract

Candida auris is a major cause of invasive fungal bloodstream infections globally and has been categorised as a critical priority pathogen by the World Health Organization, due to its persistence in hospital settings and propensity for multi-drug resistance. Recommended treatment for *C. auris* bloodstream infections is echinocandin therapy, as only ~2% of *C. auris* isolates are resistant. However, treatment can still fail despite isolates being susceptible according to minimum inhibitory concentration (MIC) testing. Using two isolate series from patients with a *C. auris* bloodstream infection refractory to anidulafungin (echinocandin) therapy, despite lack of classical resistance, we performed population analysis profiling (PAP), an agar dilution method, which identified subpopulations within clonal isolates displaying reduced susceptibility to the anidulafungin. Additionally, by performing PAP testing for a prolonged incubation of 7 days, we have identified two different subpopulations, one slow growing and one faster growing in the presence of anidulafungin. Further growth kinetics experiments with these two subpopulations also showed different growth phenotypes in absence of drug. Flow cytometry with calcofluor white staining showed elevated cell wall chitin content in response to anidulafungin pressure in both subpopulations, compared to the main susceptible population of cells. The presence of these subpopulations reveals heterogeneity in clonal *C. auris* populations, and provides a link between heteroresistance and failure of anidulafungin therapy. Furthermore, combination of anidulafungin and another antifungal drug flucytosine delayed the expansion of these heteroresistant subpopulations observed during exposure to anidulafungin alone *in vitro*, suggesting combination treatment may help tackle anidulafungin heteroresistance in *C. auris*.

Understanding the biological response of the fungi *Candida albicans* to a silica dioxide nanoparticle coating

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Abstract

Candida albicans is an opportunistic fungal pathogen, commonly associated with nosocomial infections, particularly those involving indwelling medical devices. To mitigate these infections, SiO_2 nanoparticle coatings have emerged as a promising approach to reduce *C. albicans* attachment, growth, and biofilm formation. This study investigates the biological response of *C. albicans* to SiO_2 nanoparticle-coated polystyrene surfaces, examining parameters such as adherence, growth kinetics, biochemical changes, and molecular responses.

Results showed that SiO_2nanoparticles-coated surfaces significantly reduced *C. albicans* adhesion, growth rate, and biofilm formation. Microscopy revealed that the yeast-to-hyphae transition, triggered by horse serum, was significantly reduced on nanoparticle-coated surfaces, with this response modulated by the specific composition of the culture media.To analyze molecular-level responses, RNA-Seq and bioinformatics tools were utilized, showing significant differential gene expression between treated and control samples. Pathway analysis highlighted upregulation in amino sugar and nucleotide sugar metabolism and galactose metabolism, while ribosomal metabolism pathways were downregulated.

FT-IR spectroscopy further indicated biochemical composition shifts, specifically a decreased protein-to-polysaccharide ratio in *C. albicans* cells exposed to SiO_2 nanoparticles, supporting the RNA-Seq data. NanoString technology was employed to confirmed the molecular and biochemical responses to nanoparticle exposure.

These findings underscore the efficacy of SiO_2 nanoparticles in disrupting *C. albicans* biofilm formation and inducing genetic regulatory changes. However, additional research is required to validate this approach as a viable strategy for controlling fungal colonization on medical devices and preventing related infections.

The role of mitochondria in stress resistance and virulence in C. neformans.

Dmytro Prasolov, Campbell Gourlay

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Abstract

Cryptococcus neoformans is an opportunistic fungal pathogen on the WHO priority fungal pathogens list and predominantly affects immunocompromised individuals, causing as many as 181,000 deaths annually. One of the hallmark features of this fungus is its ability to persist within the host in a dormant state for decades. It has been observed that as many as 70% of children in densely populated areas in the USA are exposed to this yeast. Previous literature suggests a role of altered mitochondrial morphology in a hypervirulent strain of *Cryptococcus neoformans* in an outbreak on Vancouver Island, warranting further research. In our work, we identified that an alternate oxidase aox1, which is a part of the electron transport chain, is vital for withstanding a variety of oxidative stresses faced by mitochondria. In addition, we identified several transcription factors and kinases which are important in aiding *C. neoformans* with withstanding mitochondrial stress. Furthermore, we are currently investigating the role of both mitochondrial stress and the alternate oxidase on forming titan cells. These are enlarged cells ranging up to 100 microns in size which enable the fungus to evade phagocytosis by macrophages as well as protecting it from a variety of environmental stresses. Finally, we are studying compounds that specifically inhibit fungal respiration and whether they can be used as antifungal therapies in the future.

The potential of AI for microbiology

Invited: Machine learning versus statistical inference in microbial genomics

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University of Oxford, Oxford, United Kingdom

Abstract

The advent of vast genomic datasets has transformed microbiology, presenting opportunities and challenges for data analysis. The distinct philosophies of machine learning (ML) and statistical inference gives them complementarity strengths and weaknesses in tackling big data problems in pathogen research. While statistical inference prioritizes understanding underlying relationships, ML focuses on optimizing predictive performance. In this talk I will contrast the approaches and offer a view on their relative utility for three problems: source attribution, bacterial genome-wide association studies, and predicting antimicrobial resistance phenotypes from whole genome sequences.

Invited: Machine learning in metagenomics for precision medicine and personalized nutrition

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Abstract

Machine learning is increasingly important in microbiology where it is used for tasks such as predicting antibiotic resistance and associating human microbiome features with complex host diseases. Specifically in metagenomics, machine learning is becoming a crucial tool for discovering and assessing the strength of microbiome associations with host phenotypes and for clinical applications. I will introduce the main machine learning concepts and tasks in metagenomics, with specific applications in metagenomic screening for colorectal cancer, in supporting precision nutrition approaches, in maximizing response to microbiome-linked melanoma immunotherapy response, and for guiding fecal microbiota transplantation protocols.

Invited: Genomic traits and social determinants of health drive bacterial antimicrobial resistance: current trends and projections to 2050 revealed by machine learning

Alexandre Alexandre Maciel-Guerra[1], Michelle Baker[1], Ruoqi Wang[2], Luo Chengchang[2], Yan Xue[2], Tania Dottorini[1], Kubra Babaarslan[1]

[1]University of Nottingham, Nottingham, United Kingdom. [2]University of Nottingham, Ningbo, China

Abstract

resistant (MDR) traits, mobile genetic elements (MGEs), microbiome, cross-species and multi-hosts transmission and key social determinants of health (socioeconomic, environmental, antibiotic consumption, mortality, health etc). These interconnected factors collectively shape current and future AMR trends. Understanding the intricate networks of AMR trait-factor interactions requires a broad, multi-scale observational perspective. However, the search for correlations hidden beneath such complex aggregates of multiscale, heterogeneous variables, poses a significant challenge. Conventional analytical approaches cannot cope with such complexity and scale of data, therefore more sophisticated methods are needed. Recent years have seen tremendous growth of AI-enabled methods expanding the breadth of investigation. We will explore how machine learning and predictive modelling answered pivotal questions, such as which AMR traits and MGEs, identified as potential drivers of treatment failure, will spread globally over the next 30 years, posing a global health risk, and what are the main drivers shaping these trends? We will present how machine learning has facilitated the identification of key genomic drivers, shedding light on lineage transmission patterns and disease severity markers. Furthermore, we will show how machine learning, metagenomics, and smart sensing technologies have uncovered the intricate dialogue between the microbiome and its residing bacteria.

Evaluation of Large Language Models for the reassembly of proteins from peptides

Shrutik Rakesh Kharkar, Enda Howley, Florence Abram

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Abstract

Proteomics and metaproteomics play a crucial role in biomedical, environmental and biotechnological research in the post-genomic era. While genomics and transcriptomics provide information on microbial potential, proteomics is closer to phenotypes and as such reflects well microbial functions. Proteomics typically involves peptide digestion followed by tandem mass spectrometry (MS) with protein identification relying on the efficient mapping of MS peaks. This typically requires access to relevant databases, which are not readily available for example in the case of soil. To overcome this limitation, de novo peptide sequencing can be deployed, which mines directly peptide sequence information from MS spectra. Such strategy however does not address de novo protein reassembly. Here, we propose to use Large Language Models (LLMs) to predict protein sequences from fragmented peptides. As a first step, we evaluated the ability of LLMs to assign unknown peptides to their proteins. To this end, we fine-tuned BERT, RoBERTa, ALBERT and ProteinBERT on peptide sequences and assessed the impact of artificially masked data on model performance. RoBERTa outperformed other models reaching 83% accuracy when trained on augmented data, while ProteinBERT, which was initially designed to predict proteins properties only display 3% accuracy when trained on the same dataset. Overall we demonstrate that LLMs are a promising avenue for de novo protein reassembly, when deployed with appropriate data augmentation strategies.

Development of AI-assisted pipelines to streamline functional annotation of pathogen proteins from scientific literature in UniProt

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European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus, Hinxton, Cambridge, United Kingdom

Abstract

Manual extraction of experimental data from scientific literature is crucial for generating high-quality functional annotations of proteins in the Universal Protein Resource (UniProt) database. As the volume of research describing pathogen protein functions grows exponentially, recent advances in large language models (LLMs)—a specific category of generative artificial intelligence (AI) capable of diverse natural language processing tasks—offer new opportunities to enhance the efficiency of literature-based data retrieval for manual curation and analysis.

This project aims to assess the capabilities of LLMs in extracting experimental data about protein functions directly from full-text papers, structuring this data, and assigning accurate Gene Ontology (GO) terms. GO annotation proved to be especially challenging for smaller open-source LLMs. In many instances, these models produced inaccurate GO terms with links between identifiers and names that were misaligned, obsolete or non-existent terms. However, the implementation of retrieval-augmented generation (RAG)—an approach that integrates LLMs with external knowledge sources to enhance accuracy and reliability of model outputs—significantly improved the correctness of GO terms suggested by open-source models by supplementing queries with up-to-date information from GO ontology database.

While the approach shows promising results, many challenges remain. Further research is needed to enhance the quality of LLM-generated summaries, optimize pipelines for data retrieval from external sources, and improve LLM-assisted selection of nuanced GO terms.

Acknowledgement:
"UniProt is a collaboration between the European Bioinformatics Institute (EMBL-EBI), the SIB Swiss Institute of Bioinformatics and the Protein Information Resource (PIR)."

Urogenital microbes in health and disease

Invited: Immunoregulation in the female genital tract

Lyle McKinnon

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Abstract

The mucosal barrier in the female reproductive tract often provides robust protection against acquisition of sexually transmitted infections such as HIV. However, in cases where genital inflammation is present, barrier permeability increases and immune cell influx creates an environment that can be exploited by pathogens. Many interacting mucosal variables contribute to cause inflammation, and achieving optimal immunoregulation could be important for improving reproductive health. One important determinant of the vaginal immune environment are commensal bacteria, which in the optimal state cause minimal inflammation and are refractory to HIV infection. When bacterial communities shift to non-optimal communities associated with bacterial vaginosis, inflammatory responses follow, and if prolonged, are associated with adverse outcomes. Understanding how these responses are regulated, and heterogeneity between individuals, is an important research goal. In human cohorts and mouse models, we have explored commensal-host interactions at the female genital mucosa. Tissue resident CD4+ T-cells play critical roles in regulating mucosal immune responses, with regulatory T-cells capable of restraining inflammatory responses and preserving tissue integrity. Bacteria themselves actively manipulate the chemokine response, leading to increased HIV infection risk. Genital inflammation and non-optimal vaginal microbiota play important roles in HPV persistence, and may need to be overcome in therapeutic HPV vaccines. Understanding regulation of healthy levels of inflammation may be important for improving vaginal health.

Invited: Modulation of the vaginal microbiome as a therapeutic target for preterm birth prevention

Lynne Sykes

Imperial College London, London, United Kingdom. The Parasol Foundation Centre for Women's Health and Cancer Research, London, United Kingdom. The Imperial March of Dimes Centre for Preterm Birth Research, London, United Kingdom

Abstract

Despite many targets being set globally to reduce the rate of preterm birth, no new interventions have been introduced for over half a century. This has led to zero improvements in preterm birth rates, with preterm birth still accounting for the majority of childhood morbidity and mortality cases. Although a proportion of preterm births are medically indicated, the majority follow spontaneous onset of contractions, and/or premature dilation of the cervix or preterm rupture of membranes. The most common causal association in these cases include inflammation, which is predominantly microbial driven. Despite this, there are no effective treatments that modulate the immune response or vaginal microbial composition and lead to preterm birth prevention.

Our research group, along with many others, have demonstrated the clear association between vaginal microbial composition and risk of spontaneous preterm birth. It has been recognised for decades that a pro-inflammatory response is also associated with a higher risk of preterm birth, however these studies have mostly been limited to reporting on cytokine concentrations. Our group has recently unravelled a potential role for activation of the complement system at the local cervicovaginal interface. This talk will summarise the current understanding of the role of microbial driven inflammation in modulating the risk of preterm birth, and will share new data supporting the role of live biotherapeutics as immune modulators and potential novel treatments for preterm birth prevention.

Invited: Harnessing Vaginal Microbiome Science for Women's Health: Emerging Pathogens and Their Role in HPV-Driven Cervical Cancer

Melissa M. Herbst-Kralovetz

Department of Obstetrics and Gynecology, College of Medicine - Phoenix, University of Arizona, Phoenix, USA. Department of Basic Medical Sciences, College of Medicine – Phoenix, University of Arizona, Phoenix, USA

Abstract

Cervical cancer disproportionately affects Latina and Native American women, with persistent human papillomavirus (HPV) infections and cervicovaginal dysbiosis as key drivers. Non-*Lactobacillus*-dominated microbiota, particularly *Fannyhessea vaginae* and *Sneathia* spp., have been associated with HPV persistence and cervical cancer. A systematic review of 25 studies involving 131,183 Latina women identified 42 cervicovaginal bacterial species associated with cervical health and disease, with *Sneathia* and *Fusobacterium* spp. consistently enriched during cervical carcinogenesis. Latinas exhibited higher microbial diversity, reduced *Lactobacillus crispatus*, and increased *L. iners*, regardless of HPV status. Similarly, *F. vaginae* and *Sneathia* were prevalent among Hispanic, non-Hispanic White, and Native American women with dysbiotic microbiota, correlating with elevated vaginal pH, high-risk HPV (e.g., HPV31, HPV52), and reduced *Lactobacillus* dominance. Mechanistic immunometabolic studies using human 3D cervical epithelial models demonstrated that *F. vaginae* and *Sneathia amnii* disrupted the epithelial barrier, induced proinflammatory cytokines (e.g., IL-1α, TNFα), oxidative stress, immune checkpoint proteins (e.g., PD-L1, LAG3), and altered barrier-related metabolites (e.g., mucins, sialic acid). Clinically, dysbiosis correlated with elevated pro-oncogenic metabolites (e.g., 4-hydroxybutyrate), cancer biomarkers (e.g., CEA, MIF), and cytokines. These findings identify *F. vaginae* and *Sneathia* spp. as emerging pathogens promoting HPV persistence and cervical carcinogenesis. Targeting these microbial pathways could inform microbiome-based interventions to reduce cervical cancer disparities in underserved populations.

Time to reconsider how we study the phenotypes and metabolism of uropathogenic *Enterobacteriaceae*?

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Abstract

Biofilm formation by uropathogens is usually studied using rich laboratory media, but artificial urine (AU) or human urine (HU) is nutrient-poor in comparison. In addition, the environment of the bladder is microaerobic (~5 % O_2), but routine laboratory work with uropathogens is done under aerobic conditions. We sought to understand the influence of oxygen conditions (aerobic, microaerobic) and physiologically relevant growth substrates (AU, HU) on the ability of bacteria isolated from catheter-associated urinary tract infections (CAUTIs) to form biofilms, and to begin to define the CAUTI bacterial metabolome. Our isolates represented 48 well-characterized uropathogens representing seven different genera of Gram-negative bacteria. Spent media were collected and analysed using 400 MHz ^{1}H-NMR, to quantify major metabolites present in AU and HU. There was a significant decrease in biofilm formation for all uropathogens when grown in physiologically relevant media compared with laboratory medium. Untargeted metabolomic analysis of uninoculated AU and HU showed the AU used in this study did not recapitulate the complexity of major metabolites present in HU. Further analyses of spent HU highlighted significant increases in acetate production by *Escherichia coli* and *Klebsiella pneumoniae* strains when they were grown under microaerobic compared with aerobic conditions. Growth of uropathogenic *Enterobacteriaceae* under physiologically relevant conditions (i.e. HU, 5 % O_2) generates data more relevant to clinical disease and is an important consideration for future work on bacteria causing urinary tract infections.

Macrolide resistance in Chlamydia trachomatis, Mycoplasma genitalium and Treponema pallidum in the United Kingdom: Genotypic Prevalence Study

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Abstract

The increased emergence of macrolide resistance among sexually transmitted bacteria has threatened successful treatment strategies, yet data on the prevalence of macrolide resistance these organisms in the UK remains limited. As such, the prevalence of macrolide resistance associated mutations (RAMs) in *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Treponema pallidum* in the UK were investigated and findings were compared with the literature to examine whether current assumptions on resistance prevalence were met. For *C. trachomatis* and *T. pallidum*, macrolide resistance assays comprising polymerase chain reaction and Sanger sequencing were developed and tested on clinical specimens positive for *C. trachomatis* (n = 82) and *T. pallidum* DNA (n = 87). For *M. genitalium*, a laboratory information system database search of clinical specimens received for routine macrolide resistance testing between 2019 and 2023 was conducted. Overall, macrolide RAMs were absent in all *C. trachomatis*-positive specimens available for analysis (n = 80). However, RAMs were identified in all sequenced *T. pallidum*-positive specimens (n = 86), with A2058G found in 98.8% of specimens (85/86), and A2059G only found in one. Moreover, macrolide RAMS were found in 59.14% (1,779/3,008) of all successfully sequenced *M. genitalium*-positive specimens, with A2058G, A2059G, A2058T and A2059C being the most common. Altogether, these findings were largely concordant with published findings and support UK guidelines no longer recommending single-dose macrolide treatment for *M. genitalium* and *T. pallidum* infections; also demonstrating that macrolides remain a viable option for the treatment of chlamydia, so long as the absence of *M. genitalium* co-infection is first established.

Activity of urinary extracellular vesicles against biofilm-associated urinary tract infections.

Aziz Ur Rehman

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Abstract

Urinary Tract Infections (UTIs) are the most common bacterial infections and up to 80% of infections are caused by *E. coli*. Various other bacterial species are responsible for the remainder of infections such as *P. mirabilis*, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis*, and *S. aureus*. Recurrent UTIs (rUTIs) is common and linked to the formation of biofilms. Urinary extracellular vesicles (UEVs) are small 'packages' released from all cells in the urinary tract. UEVs have been previously shown to exert antimicrobial activity, but their effect on biofilms is less understood. To investigate the activity of UEVs against biofilm-associated UTIs, UEVs were isolated from human urine by ultracentrifugation, characterised by transmission electron microscopy and proteomic analysis, and quantified by nanoparticle tracking analysis. Predetermined quantities of UEVs were then incubated with biofilms of UTI pathogens, and biofilm inhibition was monitored. We show that UEVs from some healthy volunteers prevent the formation of biofilms in a dose-dependant manner, thereby highlighting the importance of UEVs in combatting biofilm-associated UTIs.

Drivers of acquisition of antimicrobial resistance in urinary tract infections

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Abstract

Treatment of urinary tract infections (UTIs) accounts for 22% of all antibiotics used in the UK, costing £47.6 million in treatment costs. High antibiotic use drives antimicrobial resistance (AMR). However, little is currently known about what drives the acquisition of AMR in UTIs.

Here, we used a bioinformatic approach using publicly available metagenomic and whole genome sequencing datasets to determine the possible drivers of AMR in UTIs. Additionally, we used physiologically relevant environments, including a urothelial organoid which mimics the human bladder, to replicate the evolution of AMR in UTIs.

We found plasmid replicons and AMR genes which confer resistance to antibiotics recommended for the treatment of UTIs present in the urobiome of patients with no history of UTIs, with a history of recurrent UTIs (rUTIs) but in remission and with a history of rUTIs with a currently active UTI. AMR was primarily driven by the acquisition of AMR genes for cephalosporins and trimethoprim and by mutation for fosfomycin in uropathogenic *Escherichia coli*. We assessed the selection of resistance to fosfomycin in the urothelial organoid, with mutations arising in genes associated with fosfomycin resistance in UTIs, and the successful conjugation of the pOXA-48 plasmid in urine.

We identified that the drivers of AMR in UTIs is dependent on the antibiotic, with either mutation or AMR gene acquisition dominating, while the urobiome may act as a reservoir for AMR plasmids. Finally, we were able to replicate plasmid conjugation and the selection of fosfomycin resistance in a urinary environment.

Development of a human cell-based microphysiological system (MPS) to study vaginal mycobiome-host interactions.

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Abstract

Vulvovaginal candidiasis (VVC) is a fungal infection, primarily caused by *Candida albicans*, that has significant impact upon quality-of-life issues for women worldwide. It is estimated that up to 75% of women, especially those of reproductive age, will experience at least one such infection in their lifetime. Furthermore, the incidence of debilitating long-term recurrent VVC (RVVC) infections is increasing, with up to 150 million women worldwide predicted to be affected by 2030.

To date, much of the research on *C. albicans* pathogenicity during VVC has been conducted using mouse models. However, such models cannot faithfully replicate human physiology or anatomy and with their distinct mycobiomes may not properly reflect human infection. For instance, the murine vagina has a near neutral pH (pH 6.5-7.0) in contrast to the much more acidic vaginal environment in women (pH 3.5-5.0), and *C. albicans* is not a normal member of the murine vaginal microbiota.

To address this scientific shortfall, we have developed a cost-effective human microphysiological system (MPS), using vaginal epithelial cells (VECs), to accurately model vaginal-fungal infections in a physiologically relevant system and predict *in vivo* susceptibility, resistance and transmission.

Here we present preliminary results from a pilot study where we tested our human vaginal-fungal MPS with a set of clinical isolates of *C. albicans* carrying different allelic variants of the candidalysin-encoding *ECE1* gene.

Origin and evolution of plasmid-mediated beta-lactam resistance in *Neisseria gonorrhoeae*

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Abstract

Neisseria gonorrhoeae (the gonococcus*)* is a leading cause of sexually transmitted infection (STI) that has developed resistance to all first-line antibiotics. The beta-lactamase plasmid p*bla* has contributed to the ending of penicillin treatment, and could undermine the current first-line treatment, ceftriaxone. However, little is known about the origin of p*bla* and its evolutionary trajectory. We systematically searched the public database PubMLST to determine the origin and host range of p*bla.*In contrast to chromosomal resistance determinants that are acquired from commensal *Neisseria* spp., p*bla* was introduced into *N. gonorrhoeae* on at least two independent occasions from *Haemophilus ducreyi*, another STI pathogen. Whilst we observe a high plasmid prevalence in these two pathogens in the urogenital niche, the related *H. influenzae* and *N. meningitids* located in the nasopharynx show low p*bla* carriage, suggesting selective pressure for p*bla* in the urogenital niche. Phylogenetic analysis of p*bla* shows adaptation to the gonococcus mainly through gene loss, leading to a decrease in plasmid-imposed fitness costs, and an accumulation of mutations increasing resistance. Currently, beta-lactam antibiotics are the mainstay of treatment for both gonococcal and meningococcal disease. Our results highlight the continuing adaptation of p*bla* to its host, which could undermine treatment options in the future.

Multi-omic analysis of the vaginal microenvironment reveals mechanisms of vulvovaginal and sexual health symptoms in women with benign gynaecological conditions

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Abstract

Women with endometriosis, adenomyosis, and fibroids are at greater risk of experiencing vulvovaginal symptoms and sexual dysfunction; however, not all women with these benign gynaecological conditions will experience such symptoms. Our aim was to analyse vaginal microbiota and immunometabolic differences between women with varying sexual health symptom severity to decipher the potential mechanisms driving this distinction.

Metabolomes, immunoproteomes, and microbiomes were characterised from cervicovaginal lavages and vaginal swabs collected from 79 women undergoing hysterectomy for benign gynaecological conditions. Validated patient-reported sexual health surveys were also collected and used to differentiate patients by symptom severity. Integrative bioinformatic tools were utilised to analyse associations between vaginal microbiota, immunometabolic profiles, and severity of sexual health symptoms.

Downregulation of glycerophospholipids and sphingomyelins and dysregulation of fatty acid oxidation and histamine metabolism pathways were identified in women with more severe vaginal symptoms. Immune checkpoint proteins involved in T-cell regulation—PD-1, PD-L1, LAG-3—were associated with vulvovaginal symptom severity. The overall vaginal microbiota composition, measured by alpha- and beta-diversity and *Lactobacillus* dominance, did not significantly differ between severity of sexual health symptoms. However, when analysed at the species level, health-associated *Lactobacillus crispatus* was significantly depleted in women experiencing vaginal soreness, and dysbiotic bacteria, including *Sneathia amnii*, *Megasphaera lornae*, and Group B *Streptococcus*, were significantly enriched in women experiencing severe vaginal symptoms.

Our study elucidates potential key mechanisms underlying vulvovaginal and sexual health symptoms in women with benign gynecologic conditions, including a state of immune dysregulation, metabolic evidence of epithelial barrier disruption, and enrichment of dysbiotic microbes.

Teaching an old drug new tricks: exploring drug repurposing to combat chronic vaginal infections.

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Abstract

Bacterial vaginosis (BV) affects 20–30% of women of childbearing age and has profound psychological and sexual impacts on patients, including, an increased risk of miscarriage and an elevated risk of STI's. Bacterial vaginosis is a result of healthy vaginal microbiome dysbiosis (primarily *Lactobacilli* spp.), and an increase in vaginal pH. *Gardnerella vaginalis* is thought to act as the initial binding organism, and eventually, polymicrobial biofilms develop that can withstand host innate defences. Current BV treatment methods include metronidazole or clindamycin, but success rates are low (~60%) and ~40% of patients suffer from recurring infection following antibiotic therapy. Drug repurposing presents a promising avenue to explore in order to develop novel BV therapeutics. In response, we screened a commercially available drug repurposing library (1520 drugs) against both *G. vaginalis* and *L. crispatus* (at 5.5 x 10^7 CFU mL^{-1}) under anaerobic conditions. Growth inhibition studies resulted in 52 drugs that met the selection criteria (>75% growth inhibition of *G. vaginalis* and <50% inhibition of *L. crispatus*) when screened at 10 µM. Nine of these drugs are repurposed candidates and were taken forward for further evaluation, including, anti-biofilm activity and growth inhibition screening against a metronidazole-resistant (MIC: > 128 µg mL^{-1}) *G. vaginalis* isolate. All 9 repurposed candidates reduced *G. vaginalis* biofilm metabolic activity and biomass. Whilst only one of the repurposed drugs lost efficacy when evaluated against a *G. vaginalis* metronidazole-resistant isolate. This drug repurposing approach constitutes a promising strategy for the development of novel treatment regimens to combat BV.

Virus Forum: Antiviral Adaptive Immunity

Structural and immunological basis of cross-herpesvirus activity of a new humoral response induced by human cytomegalovirus vaccination

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Abstract

Human cytomegalovirus (HCMV) is a leading cause of morbidity in immunocompromised transplant patients and neonates following congenital infection, underpinning its high priority status for vaccine development. Previously we identified a novel antigenic domain (AD-6) in HCMV glycoprotein B (gB), a response elevated to the gB/MF59 vaccine and correlated with protection. Subsequently, we demonstrated that a rabbit pAb directed against AD-6 reduced HCMV cell-to-cell spread in fibroblasts as a potential mechanism for the control seen. Importantly, we now demonstrate that the AD-6 pAb potently blocks HCMV reactivation in myeloid cells – a major driver of viraemia in transplantation.

We now show that the high conservation of gB structure can be used to identify analogues of AD-6 in other HHV gBs despite minimal sequence conservation. Moreover, the pAb raised against HCMV AD-6 was also effective against HSV-1 suggesting the presence of conserved conformation-specific epitopes within AD-6. Consistent with this, a mAb raised against a linear epitope in HCMV AD-6 (which does not recognise non-HCMV AD-6s) is unable to prevent the cell-to-cell spread of HSV-1, despite being effective against HCMV.

Intriguingly, a re-analysis of the binding profiles of the HCMV-seronegative gB/MF59 vaccine recipient sera revealed a correlation between binding HCMV/HSV-1 AD-6 peptides for individual patients, demonstrating that those who generated a response to HCMV AD-6 were also able to respond to HSV-1 AD-6. These data strongly suggest that gB/MF59 generates an important humoral response directed against conformational epitopes within AD-6 and suggest targeting AD-6 is a potential vaccine strategy for multiple herpesviruses.

Whole Virion Proteomics of HCMV Reveals that gpUL141 is a Virion Component that Modulates Envelope Glycoprotein Composition to Protect Against Humoral Immunity

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Abstract

Human cytomegalovirus (HCMV) has co-evolved for millions of years with its human host, and establishes lifelong persistent infection. A substantial proportion of its 235kb genome is dedicated to manipulating host immunity through targeting antiviral host proteins for degradation or relocalisation. Quantitative proteomics of the infected cell has extensively characterised these processes, but the cell-free virion has been less well studied. We therefore conducted proteomic analysis of a clinical HCMV strain virion. This revealed multiple novel components, including the viral protein gpUL141, an NK immune-evasin that targets multiple host proteins (CD155, CD112, and TRAILR) when expressed in the cell. However, virion-delivered gpUL141 did not modulate NK-cell function. Instead, co-Immunoprecipitation of gpUL141 from virions identified interactions with viral entry glycoproteins from the trimer (gH/gL/gO), pentamer (gH/gL/UL128/UL130/UL131A), and gH/gpUL116 complexes, as well as gB. These interactions occurred via direct interactions between gpUL141 and either gH or gB. Analysis supported a model in which gpUL141 homodimers independently interacted with separate gH-containing complexes. gpUL141 encodes an ER retention domain that restricts trafficking through the ER/golgi, and limited the transport of glycoprotein complexes bound by gpUL141. As a result, gpUL141 reduced levels of multiple glycoprotein complexes on the infected cell surface as well as in the virion. This reduced syncytia formation, inhibited antibody-dependent cellular cytotoxicity (ADCC), and reduced susceptibility to neutralising antibodies. Thus, gpUL141 represents an immune-evasin that not only targets host proteins to limit NK-cell attack, but also alters the trafficking of multiple viral glycoprotein complexes in order to evade humoral immunity.

Controlling HCMV Through NK-dependent ADCC-inducing Immunotherapies

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Abstract

Human Cytomegalovirus (HCMV) can result in severe disease in immunocompromised individuals and following congenital infection. Therapeutics are limited by virus resistance and undesirable side effects, and despite many trials no vaccines are licensed. Vaccine studies have focussed on the induction of neutralising antibodies that target cell-free virus, however HCMV spreads cell-to-cell within the host, limiting the efficacy of this response. There is therefore a need for strategies targeting the infected cell directly. We investigated the capacity of monoclonal antibodies (mAbs) to bind infected cells and activate NK-dependent antibody-dependent cellular cytotoxicity (ADCC). Despite encoding immune-evasins, HCMV infected cells were susceptible to ADCC. Profiling of the infected cell-surface via quantitative proteomics identified 15 non-structural viral proteins expressed during early stages of infection, of which five activated ADCC. Human mAbs targeting one of these antigens efficiently controlled virus spread through ADCC when used as a mix of 5 against multiple epitopes. To reduce the number of mAbs required, they were converted into antibody-like constructs, including Redirected Optimised Cell Killers (ROCKs) and Antibody-based NK cell Engager Therapeutics (ANKETs), which contained enhancements to improve binding with, proliferation and activation of, NK cells. The ANKET platform has been used to generate successful anti-cancer constructs; however, although anti-HCMV ANKETs led to ADCC against cells expressing UL141 from a vector, they were unable to drive significant ADCC against HCMV. In contrast, ROCKs mediated ADCC as single constructs and strongly controlled virus spread at concentrations 100-times lower than pooled polyclonal IgG. This reveals a novel immunotherapeutic approach against HCMV.

The Impact of Viral Genomic Diversity on T cell Responses to Enteric Human Adenoviruses

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Abstract

Enteric adenoviruses are a leading cause paediatric morbidity and mortality worldwide. The cellular immune response to human adenovirus (HAdV) infection is known to ameliorate symptoms and help viral clearance, but little is known about the response to enteric adenovirus types. We hypothesised that the capsid proteins of HAdV are evolving under pressure to escape cellular immune responses.

Using 50 years of adenovirus genomes from around the world, we utilised data on HLA frequencies to predict CD8+ T cell binding sites within the hexon and penton proteins of HAdV-F 40 and 41, focusing on MHC class I alleles common in the UK.

Predicted CD8+ T cell epitopes were synthesised as 15mer peptides. The cellular IFNγ and IL2 responses to these peptides were measured in healthy blood donors using Fluorospot. We demonstrate that healthy blood donors are able to make a cytokine response to predicted epitopes, with some epitopes showing evidence of immune escape.

The hexon and penton proteins of enteric adenoviruses are predicted to contain a number of genotype-specific, but conserved, CD8+ T cell epitopes which could be used to inform future vaccine design; as well as variable sites likely to be under evolutionary pressure. We show for the hexon of HAdV-F41 that predicted variable epitopes detected in emergent strains are able to elicit an inflammatory cytokine response from healthy donor PBMC, but some novel epitopes may evade existing cellular immunity in a subset of donors. The role of T cell recognition in driving enteric adenovirus evolution deserves further consideration.

Developing Nanobodies as Pan-SARS-CoV-2 Therapeutics

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Abstract

Nanobodies are single domain antibodies, derived from heavy chain antibodies in camelids. Our collaborative research involves generating and characterising nanobodies, and investigating their potential as therapeutics against a broad range of coronaviruses. We have identified a panel of nanobodies that bind to the S2 region of the SARS-CoV-2 spike protein, and measured their binding via ELISA and biolayer interferometry (BLI). The S2 region of the coronavirus spike protein is less immunogenic, but more conserved than the highly variable receptor-binding domain, and therefore we have tested these nanobodies against a wide of SARS-CoV-2 variants of concern (VOC), using a micro-neutralisation assay. Several of these nanobodies are highly neutralising, with IC50 values in the low nanomolar range, despite the fact that S2-binding antibodies are traditionally considered to be weak neutralisers. Epitope mapping using structural biology and *in vitro* escape studies suggest key S2 protein residues that are involved in this potent neutralisation. Work is now progressing on characterising effective MERS-CoV S2-binding nanobodies, with the aim of being more prepared for potential future coronavirus spillover events.

Assessing SARS-CoV-2 viral escape under immune selection from neutralising antibodies and polyclonal serum.

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Abstract

The ongoing viral escape through the evolution of SARS-CoV-2 omicron subvariants and the threat of potential spillovers of future sarbecoviruses has driven the continuous search for broadly neutralising therapeutics. Evaluating the risk of viral escape under immune selection is vital in determining the efficacy of broadly neutralising antibodies against evolving variants of SARS-CoV-2.

Employing passage studies and next generation sequencing (NGS), we evaluate the risk of escape from neutralising antibody and potent polyclonal serum. *In vitro* passaging under immune selection provides a controlled environment to investigate new mutations, thus potentially identifying mutations that may confer a selective advantage. SARS-CoV-2 variants were serially passaged with increasing concentrations of neutralising antibodies or polyclonal serum and assessed by microneutralisation assays comparing passaged virus with the wild-type variant. Viruses that reduced neutralisation were sequenced and analysed.

In under ten passages, a recent SARS-CoV-2 omicron subvariant was able to either fully or partially escape neutralisation from tested antibodies by gaining mutations within both the S1 and S2 of the spike. To achieve escape from potent polyclonal serum, a greater number of passages with lower concentrations of serum were required. Further investigations are required to determine the fitness of these mutant viruses. Our studies demonstrate that the spike, including the conserved S2, is capable of mutation under selective pressure. This may aid in predicting viral evolutionary dynamics, and in informing the response to future pandemics, public health strategy and vaccine design.

Understanding rotavirus vaccine efficacy by analysis of antibody responses in mice

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Abstract

The recent failure of an advanced new rotavirus vaccine candidate in a phase III trial has shown how little we understand about immune responses to rotavirus. This virus is a major cause of gastroenteritis in young infants across the globe, but has been brought under control in high-income countries due to effective live attenuated vaccines. In low-middle income countries however, these vaccines are often <50% effective. This drives the need for improved rotavirus vaccine development and improved knowledge of protective immunity.

Pre-clinical models are essential for trialing new vaccine approaches, but there are two major issues with using mice to study human rotavirus vaccines. The first issue is that human rotavirus strains do not replicate well in mice. To address this, we generated chimeric rotaviruses that readily replicate in mice, but contain key immunogenic proteins from human strains. We showed that mice infected with these chimeric viruses make a robust antibody response to human rotavirus proteins.

The second issue of using mice to model rotavirus vaccination is that whereas lab mice are born immunologically naïve, human infants are born with a full repertoire of antibodies transferred from their mothers. To model this, antibodies were induced in or delivered to dams, and transfer of maternal antibodies to pups was observed. Surprisingly we found that maternal antibodies completely abolished seroconversion to rotavirus vaccination in pups. Using flow cytometry, antibody receptor knockout mice and single cell sequencing we demonstrated that maternal antibody-mediated vaccine clearance is the key mechanism of vaccine failure in mice.

A multi-dimensional model of HIV-1 latency toward assessment of effectiveness of antigen presentation and curative therapies

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Abstract

Determining factors of HIV-1 viral latency establishment, maintenance and reactivation remain poorly understood. This poses a hindrance to the “shock and kill” cure strategy, which proposes pharmacologically induced transcription of the latent provirus and subsequent death of the reactivating host cell by apoptosis or cellular effector mechanisms. To investigate this and create an improved model of the diverse reservoir of latently infected cells that persist in people living with HIV (PLWH), we generated 56 unique, latently infected Jurkat T-cell clones, each harbouring a single, full-length HIV-1 proviral genome. The model makes use of an EGFP reporter inserted into the variable loop 5 of HIV-1 glycoprotein Env, which allows both the quantification of latency reversal and direct tracking of Env subcellular localisation and cell surface presentation. This constitutes an improvement over extant cell culture models of HIV-1 latency, which are often monoclonal and rely on simple reporter systems. Using a probe-based DNA capture sequencing approach, we determined the full proviral sequence and mapped integration site in the host genome for each clone. The effectiveness of different latency reversal therapies varies significantly for each clone and, likely, depends on the unique integration site. Additionally, effective Env antigen presentation on the cell surface, a crucial factor in facilitating immune clearance of infected cells, is similarly variable and not necessarily predictable by overall reactivation. As such, we present a novel model of HIV-1 latency that allows assessment of therapeutic strategies while monitoring antigen presentation and better recapitulating the diverse reservoirs that persist *in vivo*.

Live-attenuated SARS-CoV-2 vaccine viruses as a lower containment model for coronavirus biology

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Abstract

In the five years since SARS-CoV-2 emerged, it has been responsible for ~800 million confirmed cases and over 7 million deaths. The COVID-19 pandemic has accelerated investment and increased the scope of research into coronavirus biology; however, many fundamental questions could be pursued in a safer manner using attenuated viruses.

We have adopted live attenuated vaccine viruses for SARS-CoV-2 for use as a safer alternative to live SARS-CoV-2, which present an attractive option to understand mechanisms of live-attenuated vaccine function as well as mechanisms of virus biology, while working at lower containment.

We developed in vitro reverse genetics system for attenuated SARS-CoV-2 systems, utilising in vitro assembly and direct transfection of assembled DNA. Our plasmid-based method allows reliable introduction of updated antigens and tags, and adaption of our attenuated system to study variants of concern at CL2.

Using this approach, we have generated a range of attenuated viruses with fluorescent markers and performed comparisons with competent SARS-CoV-2 viruses. We benchmarked the performance of these attenuated viruses in antiviral and neutralisation assays against wild-type SARS-CoV-2, demonstrating the utility of these live-attenuated vaccine viruses as models for studying SARS-CoV-2 biology.

Evaluation of bioinformatically designed glycoproteins to aid the development of a pan-lyssavirus vaccine

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Abstract

The lyssavirus genus comprises 18 distinct viral pathogens, all capable of causing rabies - an invariably fatal disease responsible for over 59,000 human deaths annually. Classified based on genetic and antigenic properties into three phylogroups, current rabies vaccines confer protection only against viruses within phylogroup 1. Given antigenic cross-reactivity is limited to individual phylogroups, the development of a pan-lyssavirus vaccine antigen would be highly beneficial.

Humoral responses that prevent productive infection target the lyssavirus glycoprotein (G), which share high sequence similarity within phylogroups that decreases with the greater genetic distance between phylogroups. In an approach that has proven effective for other viral species, genetic sequences of historic lyssavirus G were computationally predicted, with unknown antigenic properties.

By analysing publicly available lyssavirus G sequences, a panel of 15 predicted historic G was created and used to generate pseudotyped lentiviruses. From this panel, nine G could be pseudotyped to high titre so consequently their neutralisation profiles were assessed alongside pseudotype viruses expressing a current vaccine isolate G using antisera specific for lyssavirus species covering all phylogroups. The remaining G variants were evaluated with the same sera using binding assays, in which they were expressed on the surface of BHK cells and antibody binding analysed by flow cytometry.

Results show that the historic G exhibit different patterns of cross-reactivity, with two showing significantly higher antibody neutralisation/binding than the other G and current vaccine isolate G. This suggests they could be suitable candidates for use as pan-lyssavirus vaccine antigens and therefore require further assessment.

Changes in population immunity reduce the likelihood of emergence of zoonotic coronaviruses

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Abstract

Identifying animal coronaviruses with outbreak potential is key for pandemic preparedness. Here, we examine how SARS-CoV-2 natural and vaccine-derived cross-immunity would impact the future emergence of an unknown coronavirus (SARS-CoV-X), using a panel of SARS-CoV-2 related animal coronaviruses and SARS-CoV-1 as a natural model system. We show that both vaccination and natural infection with SARS-CoV-2 generates cross-neutralising antibodies against the spike protein of each virus in our panel, and that the levels of cross-neutralisation vary by virus and the route of immune acquisition. Mathematical simulations show a significant reduction in the likelihood of SARS-CoV-X emergence in populations with co-circulating SARS-CoV-2, with the main factors driving this reduction being: i) the level of cross-immunity elicited by vaccines and natural infection; ii) vaccination coverage; and iii) the effective reproductive number of SARS-CoV-X. Due to knock-on effects on SARS-CoV-2 prevalence, the emergence of SARS-CoV-X was more likely in simulations with vaccination than those without vaccination. Nevertheless, our findings show that the current levels of vaccination and SARS-CoV-2 circulation present a powerful barrier against the emergence of novel animal coronaviruses.

Virus Forum: Antiviral Innate Immunity

Assessing the anti-influenza activity of equine ISGs

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Abstract

The respiratory tract of the horse has receptors for avian influenza A viruses (IAVs), and previous studies have shown that they are regularly exposed to avian IAVs. Nevertheless, only one equine influenza virus (H3N8 EIV) circulates.

The aim of this project is to identify equine genes with anti-IAV activity. To this end, we generated a library of 288 equine IFN-stimulated genes (eISGs). These were transduced in equine lung cells, which were further challenged with IAVs of equine, avian, and human origin. For eISGs that displayed anti-IAV activity, we determined their level of expression in the respiratory tract of the horse using *in situ* hybridization. Additionally, we generated ISG-overexpressing cell lines, to further test the inhibitory effect of equine ISGs *in vitro.*

In transient transduction experiments, some eISGs exhibited virus-specific restrictions: RAPGEF3 was more potent against human H1N1 IAV than H3N8 avian IAV, while SHISA5 was effective against avian-like H3N8 subtypes. IFIH1 was observed as a broad antiviral eISG that inhibited both subtypes similarly. In stable transduction experiments, only IFIH1 restricted IAV infection, reducing titres of H1N1 and H3N8 subtypes up to twofold.

IFN-treated equine tracheal explants displayed higher expression of inhibitory eISGs. However, in EIV-infected tissues only SHISA5 and IFIH1 were overexpressed.

In summary, we found that equine IFIH1 is an important component of the equine host barrier against multiple influenza A viruses, which could aid the design of novel antiviral strategies to control influenza emergence.

Species differences in IFIT sequence, interaction and function

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Abstract

The interferon (IFN)-induced proteins with tetratricopeptide repeats (IFIT) are a family of IFN-stimulated proteins involved in viral restriction. These proteins are differentially expressed between species, and whilst they have been studied extensively in humans and mice, there is little knowledge of the IFITs in viral reservoir species. We aimed to interrogate the mechanisms responsible for antiviral activity of different IFITs in a reservoir bat species, *Pteropus alecto* (*Pa*-black fruit bat).

We previously showed that human IFIT1 binds to RNA containing a 5' end cap0 structure. Cap0 RNA, which lacks 2'-O-methylation of cap-adjacent nucleotides, is sensed as 'non-self' RNA, and is susceptible to IFIT1 translation inhibition, compared to 2'-O-methylated cap structures (cap 1 and 2). Interaction of human IFIT1 with IFIT3 also enhances the antiviral effects of IFIT1 *in vitro.* Using similar *in vitro* reconstitution methods, we demonstrate that *Pa*IFIT1 inhibits translation of cap0 mRNAs bearing the 5' regions with weak RNA secondary structure including that of SARS-CoV-2, while RNAs with more stable secondary structures are more resistant to *Pa*IFIT1 inhibition. We also previously identified a conserved motif present in both human IFIT1 and IFIT3 allowing for their interaction. Despite sequence differences in this conserved motif from humans, *Pa* IFIT1/IFIT3 oligomerisation is unaffected. *Pa* IFIT1/IFIT3 oligomerisation was also found to enhance translation inhibition of viral mRNA with strong secondary structure at the 5'-end.

Alternative splicing diversifies the antiviral activity of OAS proteins

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Abstract

The emergence of SARS-CoV-1, MERS-CoV and SARS-CoV-2 in recent decades highlights the importance of understanding immune barriers to coronavirus infection. One such barrier is the interferon response, which triggers the upregulation of interferon-stimulated genes (ISGs) that can exhibit specific and potent antiviral activity. Arrayed ISG expression screening with a library of >500 human, >300 macaque and >250 bovine genes revealed oligoadenylate synthetase 2 (OAS2) as an inhibitor of the endemic betacoronavirus HCoV-OC43. The antiviral activity of ectopic OAS2 expression, confirmed at the level of viral transcript, protein and replication, was found to be isoform specific. OAS proteins classically activate RNase L via synthesis of 2'-5'-oligoadenylate, resulting in degradation in cellular and viral RNA. However, HCoV-OC43 encodes a 2'-5'-phosphodiesterase that can antagonise this pathway, suggesting a RNase L-independent mechanism. Mutating catalytic residues of OAS2 and depletion of RNase L support an alternative mechanism of restriction by this ancient protein. Understanding these mechanisms by which ISGs specifically inhibit coronaviruses provides new information on interactions of both endemic and emerging coronaviruses with the innate immune system.

Utilising spatial proteomics to understand the cellular response to IFN

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Abstract

Cellular sensors and signalling components form the first step in the recognition of intracellular pathogens, and determine the outcome of infection by orchestrating an effective innate immune response. Notably, many signalling proteins exhibit relocalisation upon activation. These include the transcription factors IRF3 and NF-kB, which trigger expression of interferon and antiviral proteins. The discovery of related, pathways will provide new insights into our understanding of innate immunity and the antiviral response.

We have developed an innovative proteomic approach to identify changes in subcellular protein distribution upon any infectious or chemical stimulus. Human fibroblasts were subjected to type I interferon (IFN) stimulation followed by temporal quantitative proteomics alongside sub-cellular fractionation and analysis of protein relocalisation on a global scale.

Key mediators of the IFN response, such as IRF9 and STAT1/2, were shown to translocate into the nucleus in response to IFN stimulation indicating that our methodology was robust. Surprisingly, we also observed a significant global translocation of mitochondrial proteins upon IFN stimulation, particularly mitochondrial inner and outer membrane proteins. The link between mitochondrial architecture and function is well understood, indeed mutations in proteins forming the mitochondrial membranes have been linked to a number of neurodegenerative diseases.

By combining advanced proteomics with cell biology our data has created a comprehensive subcellular protein map of human fibroblasts in basal and IFN-stimulated conditions. The remodelling of mitochondria in response to IFN is novel, and our ongoing investigations of how this affects both function and size may reveal important new facets of innate antiviral immunity.

Interleukin-1β induces ISG expression and restricts MPXV via IFITMs

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Abstract

Inflammatory and antiviral immune responses are integral host responses to virus infection. Monkeypox virus (MPXV) is a rodent zoonotic orthopoxvirus (OPXV) that re-emerged globally in 2022. To start understanding host responses to MPXV, we and others have carried out transcriptome analysis from primary human fibroblasts (HFFFs) infected with endemic or global lineages of MPXV and observed a potent inflammatory signature. This response was absent with other OPXVs. To study the biological effects of this signature, we treated HFFF, nTERT and monocytic (THP-1) cells with inflammatory cytokines including IL-1β, IL-6, IL-8, IL-11 and LIF and measured transcriptional responses. Interestingly, IL-1β induced expression of interferon stimulated genes (ISG), similar to, but to a lower extent than, type I interferon (IFN). Using flow cytometry and classical virology, we showed that MPXV and prototypic OPXV vaccinia virus (VACV) were restricted in HFFFs treated with IL-1β and, as expected, IFNβ. Blocking ISG induction with a JAK inhibitor rescued viral replication in IFNβ-, but not IL-1β-, treated HFFFs, suggesting non-canonical pathway activation. In agreement, qPCR and western blotting revealed that, relative to IFNβ, IL-1β only induced expression of a subset of ISGs including IFITMs. Finally, restriction assays showed that IFNβ and IL-1β treatment significantly affected virus focus formation, but not virus spread, and this was modulated by IFITMs. These findings demonstrate that IFITMs have the capacity to restrict OPXV and are induced by IL-1β as well as IFN, highlighting the role of these crucial cytokines during poxviral infection and questioning the roles of other pro-inflammatory cytokines.

Molecular determinants of Influenza A virus escape from human BTN3A3 restriction

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Abstract

Human BTN3A3 (butyrophilin subfamily 3 member A3) is an interferon-stimulated gene restricting the replication of avian influenza A viruses by inhibiting their viral genome replication. Sensitivity to BTN3A3 is primarily determined by the viral nucleoprotein (NP). Most avian influenza viruses are susceptible to BTN3A3 restriction, while human seasonal viruses and most zoonotic avian viruses are resistant to BTN3A3. Resistance or susceptibility to BTN3A3 maps primarily to NP residues 313 (F= susceptible, Y/V= resistant) and 52 (N/H/Q= resistant or Y= susceptible). These mutations highlight the evolutionary pressure exerted by BTN3A3 on influenza A virus (IAV). In this study, we explored additional determinants of IAV sensitivity or resistance to BTN3A3. We identified further NP residues, beyond positions 52 and 313, that influence viral escape from BTN3A3. Notably, NP from the Hong Kong H3N2 pandemic strain (1968) and a zoonotic H3N2 strain from 1999 did not follow the expected resistance pattern associated with mutations at residues 52 and 313. In these cases, resistance was conferred by a patch of NP amino acids rather than a single point mutation. Additionally, a recent H5N1 strain from Scotland (2021) exhibited BTN3A3 resistance despite having a genotype (52Y and 313F) typically sensitive to BTN3A3. In this case, a mutation at NP position 51 (N51D) adjacent to positions 52 and 313 was responsible for resistance. Understanding the molecular mechanisms underlying BTN3A3 escape in avian IAVs is crucial for assessing their zoonotic potential and strengthening global pandemic preparedness.

Functional Variation in Antiviral Activity Between Mammalian Type III Interferons

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Abstract

Respiratory viruses, such as influenza A virus (IAV), coronaviruses, and paramyxoviruses, pose major "one health" threats, especially in the absence of effective, broad-spectrum clinical interventions. Between host species, infection outcomes can vary from asymptomatic to fatal. Understanding the determinants of such divergent outcomes may help mitigate the burden of respiratory diseases by facilitating the development of novel interventions.

The innate immune response is crucial in defending against emerging infections. By inducing the expression of antiviral genes, type III interferon (IFN) 'lambdas/λ', play a critical role in controlling replication in barrier epithelial tissues like the lung targeted by respiratory viruses. *In silico* screening revealed the diversity of IFNλs, with mammals having two distinct lineages: IFNλA (IFNλ4-like) and a highly diverse IFNλB group (IFNλ-3-like). However, the phenotypic consequences of this diversity are poorly described.

Here, we measure the antiviral activity of IFNλs from three placental mammals (humans, pigs and bats) in a range of cell lines and virus infection models, including IAV in human and pig epithelial cells. These results revealed that on top of their genetic diversity, there is also considerable functional diversity in their antiviral activity, which is dependent on species origin of cell lines. Mechanistic studies suggest activity differences are not explained by changes in their relative abundance. Ongoing work seeks to investigate the molecular structural variation underpinning cytokine-receptor interactions and co-evolution underpinning IFNλ antiviral potency.

Non-CDN STING agonists are resistant to poxin antagonism and potently restrict MPXV infection

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Abstract

Monkeypox virus (MPXV) is a zoonotic virus endemic to Africa that has recently spread globally, becoming the first human orthopoxvirus since smallpox eradication. At present two antivirals are available however these have excessive toxicity and/or low barrier to viral resistance mutation. A greater understanding of how MPXV interacts with the human host, particularly innate immunity that acts as an important barrier to zoonotic infection, is greatly needed to aid the design of novel therapies. Here we show that the clade IIb 2022 MPXV outbreak strain has significant capacity to avoid innate immune detection in human cells and actively suppresses interferon (IFN)β induction by both DNA and RNA agonists. For evasion of DNA sensing this is achieved by MPXV viral Schlafen that contains a poxin domain highly conserved with vaccinia virus (VACV) poxin, a viral antagonist and critical virulence factor that cleaves cGAMP. Depletion of poxin expression during infection significantly reduced the capacity of MPXV to block DNA sensing, including STING and IFN regulatory factor (IRF)3 phosphorylation. Non-cyclic dinucleotide (CDN) STING agonist diABZI is a potent activator of IFN and is currently being tested as a novel cancer therapeutic. We hypothesised that poxin would be ineffective at antagonising diABZI and indeed found that VACV was unable to block diABZI-induced STING/IRF3 phosphorylation and IFN induction. As such, diABZI had potent antiviral activity against both VACV and MPXV, dependent on STING, IRF3 and the type I IFN receptor. Non-CDN diABZI therefore offers great promise as a novel host-directed MPXV therapeutic.

The antiviral functions of avian orthologues of viperin against IAV

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Abstract

Highly pathogenic forms of avian influenza A virus (IAV) typically cause severe disease in chickens but asymptomatic/mild disease in ducks. Previous transcriptomic studies have linked the interferon-stimulated gene RSAD2/viperin to this differential disease severity. Human viperin possesses antiviral activity in part through its radical-SAM domain. This domain catalyses the production of 3'-deoxy-3',4'-didehydro-CTP (ddhCTP), which acts as a chain terminator of flavivirus polymerases and inhibits global cellular translation.

We investigated the anti-IAV activity of chicken and duck viperin orthologues using ribonucleoprotein reconstitution assays (minireplicons). In mammalian and avian cell lines, both orthologues significantly reduced IAV gene expression, with the most pronounced inhibition caused by duck viperin. Despite different strains of virus exhibiting varying sensitivity to viperin-mediated restriction, duck viperin consistently showed the greatest inhibitory effect.

We performed minireplicon assays using viperin orthologues with mutated radical-SAM domains or in the presence of ddhCTP, which suggested that viperin enzymatic activity is unlikely to contribute to the inhibition of IAV polymerase activity. Chicken and duck viperin were further shown to carry out radical-SAM-dependent repression of cellular protein synthesis, as previously shown for human viperin. However, no differences in this shutoff activity were observed between viperin orthologues.

Overall, we find that both chicken and duck viperin possess anti-IAV activity, which is not solely dependent on the enzymatic function nor the translational shutoff ability of the protein. Duck viperin consistently showed greater anti-IAV activity than its chicken counterpart, suggesting that it may indeed contribute to the greater resistance of ducks to IAV.

Exogenous DNA and not viral genomes triggers IFN production during in vitro infection with Human cytomegalovirus.

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Abstract

The innate immune response is a fundamental cellular defence mechanism against viruses. This response is regulated by pattern recognition receptors (PRRs) that identify specific pathogen associated molecular patterns. *In vitro* infection of cultured cells with Human cytomegalovirus is known to induce robust interferon (IFN) production, with previous studies having identified the cytoplasmic double-stranded DNA sensor, cyclic GMP-AMP synthase (cGAS), as the key PRR responsible this response. Nevertheless, the question remains as to how cGAS recognises HCMV infection, as the viral genome is shielded by the capsid during transit through the cytoplasm following cell entry.

Here, we demonstrate that pre-treatment of HCMV viral stocks with DNase prior to infection of human primary fibroblasts completely abrogates production of IFN, without impacting virus infectivity. This suggests that *in vitro* induction of IFN following infection of cells is from cGAS detection of exogenous DNA, likely a contaminant from lab-generated viral stocks, rather than recognition of the viral genome. However, DNase treatment did not increase HCMV replication despite the loss of IFN production. Additionally, cGAS knockdown increased virus replication despite pre-treatment of stocks with DNase, indicating that cGAS still exerts antiviral effects independent of IFN signalling.

Further studies will be required to understand the antiviral mechanism of cGAS, and whether this relies on the detection of incoming virus.

Virus Forum: Biochemistry of Viral Infection

Structural and functional characterisation of the SARS-CoV-2 Envelope ion channel

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Abstract

SARS-CoV-2 remains a significant global health threat, particularly to vulnerable populations at high risk of severe disease and mortality, while posing a persistent risk of both acute and long-term sequelae across broader demographics due to extensive viral variation. There is a critical need for novel antiviral agents that target unique sites beyond traditional enzymatic mechanisms. The Envelope (E) protein of SARS-CoV-2, which forms an ion channel, serves as an attractive drug target due to its essential role in virulence and its involvement in various stages of the viral lifecycle. Mutations in the E protein identified in recent Omicron variants further suggest its potential role in viral transmission and pathogenesis.

Despite its significance, the structure and function of oligomeric E complexes remain incompletely understood, particularly concerning channel stoichiometry. Current models using lipid-embedded or micelle-based approaches have limitations, with some lacking complete sequences or raising structural validity concerns. To resolve these ambiguities, we have analyzed both truncated peptides and full-length recombinant E protein, including Omicron-associated T11A and T9I polymorphisms, using lipid and micelle environments. Experimental approaches included native and cross-linking SDS-PAGE and electron microscopy to elucidate the effects of protein and membrane contexts on channel stoichiometry. In parallel, we evaluated channel gating, ion selectivity, and drug sensitivity through indirect in vitro assays and electrophysiology. Our findings provide essential insights into lipid-compatible full-length E protein structure, informing targeted antiviral development strategies.

HCMV microprotein UL2 indirectly affects formation of viral biomolecular condensate compartments.

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Abstract

Human cytomegalovirus (HCMV) is a widespread pathogen causing life-altering conditions in immunocompromised individuals. HCMV contains many genes yet to be characterised. These include those within the UL2-11 region, which contains the highly conserved 6.7 kDa microprotein UL2. Here, we characterised UL2 and determined its function during infection. Experiments with a single gene KO mutant of UL2 in comparison to wildtype virus were performed to investigate the effect on viral replication in the presence or absence of UL2. The absence of UL2 did not impact viral replication. Infections with the UL2-KO mutant and wildtype viruses were performed to examine temporal expression of HCMV proteins by western blotting. There was no reduction in the expression of immediate-early or early proteins, but a decrease in the expression of the late protein pp28 (UL99) in adult fibroblasts in the absence of UL2 was observed. To characterise this further we performed yeast two-hybrid assays and confocal microscopy to investigate the interaction between UL2 and pp28. There was no direct protein-protein interaction between UL2 and pp28 in yeast two-hybrid assays. However, the confocal microscopy showed an increase in the number of assembly biomolecular condensate compartment complexes of which pp28 is an integral component. Overall, we find the absence of UL2 in infected cells decreases pp28 in cell lysate and, conversely, increases assembly compartment formation in intact cells. Although more understanding is required regarding pp28 detection using different methods, our findings represent a novel observation wherein a viral microprotein indirectly affects formation of viral biomolecular condensate compartments.

Biochemical characterisation of genome packaging interactions in coronaviruses.

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Abstract

For all viruses, specifically packaging their genome into progeny virions is crucially important for efficient transmission between hosts. This process must be tightly regulated to prevent spurious incorporation of defective viral genomes, subgenomic RNAs or host nucleic acids, resulting in non-infectious particles. Many viruses encode specific packaging signals (PS) which interact with viral structural proteins during particle assembly. While genome packaging in human coronaviruses is still poorly understood, PSs have been identified in model coronaviruses from mouse and swine.

Here, we characterised interactions between the PS and viral structural proteins in Mouse Hepatitis Virus (MHV) and its close relative, human coronavirus OC43. Purified virions were analysed by high throughput RNA structure mapping, and cross-linking and immunoprecipitation (CLIP) of the viral Nucleocapsid (N) and Membrane (M) proteins, to examine protein binding sites to nucleotide resolution. We observe striking peaks of interaction between N and PS-flanking sequences, while M binds preferentially within the 5’ and 3’ terminal regions.

We also noted interactions between N and unpaired purines along the 3’ stem of the PS, which are not bound in a mutant virus in which the PS structure has been disrupted. To confirm the role of these residues in packaging, as well as mutationally characterise other elements, we developed a virus-like particle system for OC43. In this system, we recapitulate packaging with high efficiency and confirm the role of the unpaired purines in PS recruitment. Together, this work provides a biochemical foundation for understanding the protein-RNA interactions involved in coronavirus genome packaging.

Proteolytic cleavage orchestrates the formation and function of the astrovirus replication complex

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Abstract

Astroviruses are positive-sense, single stranded RNA viruses infecting both avian and mammalian species. Classical human astroviruses are common enteric pathogens, affecting young children and presenting mild gastroenteritis symptoms. Recently, two novel non-classical strains of human astroviruses, MLB and VA, were shown to possess extraintestinal pathogenicity and demonstrate neurotropic features, with reported infections of the central nervous system. The replication cycle of astroviruses begins from the translation of a non-structural polyprotein (nsP1a) produced from the VPg-linked genomic RNA. The viral serine-like protease cleaves the polyprotein to release proteins for genomic and subgenomic RNA replication to initiate structural protein synthesis. Cleavage sites for nsP1a have been predicted but not experimentally confirmed and the subsequent processing products have not been characterised. We map the cleavage sites to study the processing dynamics in HAstV1- and MLB2-infected cells. Employing mass spectrometry-based N-terminomics, we identify novel processing sites and confirm some of the computationally predicted sites. We validate these findings by overexpressing nsP1a with processing site mutants and detecting the resulting cleavage products using the custom antibodies against the virus protease, VPg and p20 protein. We confirm the importance of the identified cleavage sites to viral replication in the HAstV1 and MLB2 replicon systems. This work advances our understanding of astrovirus non-structural polyprotein cleavage and may inform future drug targets and vaccine candidates.

Assessing the internal genes of H5N1 in bovine cells

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Abstract

In March 2024, H5N1 highly pathogenic avian influenza (HPAI) of the 2.3.4.4b lineage (B3.13 genotype) was first detected in a dairy cattle farm in the United States and has since spread to hundreds of herds across multiple states. It is therefore critical to understand the molecular determinants that have allowed H5N1 to adapt to this new host. In this study, we examined the contribution of the 'internal' gene segments of H5N1 B3.13 to mammalian adaptation. We rescued 6:2 viruses harbouring the HA and NA genes from A/Puerto Rico/8/1934 and the remaining segments from IAVs of various origins, including ancestral European avian derived H5N1. The recombinant B3.13 viruses possessed a faster replication kinetics relative to other IAVs in bovine cells. Furthermore, replication assays with viruses possessing segment swaps between ancestral European 2.3.4.4b and B3.13, suggest that no single genome segment of the latter provides a dominant advantage in bovine cells. Importantly, the B3.13 internal genes were less susceptible than an ancestral 2.3.4.4b strain to type I IFN. Both bovine and human Mx1 restricted replication of recombinant viruses with the internal genes from either B3.13 or ancestral 2.3.4.4b, and we detected expression of Mx1 in infected udder tissues *in vitro*. However, B3.13 and ancestral avian 2.3.4.4b viruses escape restriction from human BTN3A3. Finally, recombinant B3.13 was virulent in mice, unlike the ancestor 2.3.4.4b virus. Thus, some of the internal genes of B3.1.3 have adapted to the bovine host and provide a replicative advantage in cells from this species.

There is more to the Nucleocapsid gene of coronaviruses that meets the eye...

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Abstract

The second frame of the Nucleocapsid (N) gene of many coronaviruses encode important accessory genes involved in regulating virus-host interactions, which are expressed through ribosomal leaky scanning.To ask whether this is a general feature of coronaviruses, we developed a computational pipeline to identify accessory genes in the second frame of coronaviruses' N gene. This pipeline identified and analysed open reading frames' (ORFs) lengths, genomic coordinates, and likelihood of translational initiation in 113 coronavirus species from all genera. Results showed that the 2nd reading frame of the N gene of β- and δ-Coronaviruses is a reservoir for accessory genes. At the sub-genus level, translational initiation/termination sites in N's 2nd frame are conserved across related viruses, but introduction of premature Stop codons is a common cause of accessory gene variability. Human-infecting coronaviruses MERS-CoV, HCoV OC43 and NL63 had truncated ORFs compared to their other sub-genus members, but temporal phylogenetic analyses of MERS-CoV showed that its accessory gene is evolving into an ancestral longer form conserved in other merbecoviruses. Transient expression of merbecoviruse' N gene in human cells confirmed expression of their respective candidate accessory proteins. Functional characterisation of these proteins showed that these partially localise to mitochondria and are interferon antagonists, similarly to related sarbecoviruses' N-encoded accessory genes. Overall, our pipeline shows that a functional overlapping ORF in the N gene is a widespread feature of β- and δ-coronaviruses that may have evolved after a split from an α-coronavirus ancestor.

Understanding enterovirus capsid uncoating by the identification and characterisation of a highly conserved capsid motif

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Abstract

Enteroviruses (EVs) are a genus of human and animal pathogens in the picornavirus family, which pose a significant threat to global health. Despite the global importance of EVs, the molecular details of capsid uncoating and genome release are not fully understood.

Based on an analysis of over 6000 whole genome sequences of EVs, we identified a highly conserved sequence motif in the capsid protein VP0/VP4 and explored its role by creating mutations. Utilising mutant viruses recovered from *in vitro* transcribed RNA, we assessed the effect of these mutations on virus uncoating and particle formation. We have also used viral evolution studies to select second site mutations associated restoration of viral fitness. We established time-resolved uncoating assays, utilising fluorescent reporter viruses generated by trans-encapsidation. Using these approaches, we have identified residues which appear to affect the stability of EV virions and to control uncoating in a pH-dependent manner. Furthermore, high resolution structures of these stabilised intermediates are providing invaluable insight into the molecular mechanism of EV uncoating.

Together, these data have allowed us to identify critical interactions and possible metastable intermediates in the virus uncoating pathway. In addition, understanding the early stages of virus uncoating during EV infection will direct the design of antiviral compounds against all EVs.

Foot-and-mouth disease virus infection induces the assembly of biocondensates

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Abstract

Foot-and-mouth disease virus (FMDV) is highly contagious and circulates among a broad range of wild and domestic cloven-hoofed animalsuch as cattle and pigs, being a major threat to livestock worldwide. FMDV belongs to the *Picornaviridae* family of non-enveloped viruses with a positive-sense single-stranded RNA genome. A fundamental gap in knowledge for high consequence viruses such as FMDV is understanding how the virus evolved to evade or dampen cellular antiviral responses. Upon an RNA virus infection, the detection of viral components can limit viral protein synthesis, promoting the assembly of cytoplasmic membrane-less condensates, such as stress granules (SGs); and result in the activation of the RIG-I like receptors (RLR)-mediated innate immune response (IIR) to trigger the induction of antiviral genes. The FMDV leader protease has been shown to antagonize both SGs assembly and the RLR-mediated signalling, suggesting that both pathways are initiated upon FMDV infection to limit viral replication.

Herein, we characterized the dynamics and interplay of biocondensates and IIR induction during FMDV infection. First, we engineered the porcine kidney epithelial (PK-15) cell line to allow tracking of the activation of these pathways using live-cell microscopy. Our data shows that wild-type FMDV results in the transient assembly of biocondensates early during infection, while in contrast, FMDV engineered to lack the leader protease induces the formation of distinct biocondensates both in infected and bystander non-infected cells. Understanding the relevance of these condensates for the cellular IIR response will help to uncover novel potential therapeutic targets to prevent FMDV infection.

Unique structural and compositional features of Influenza A filaments

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Abstract

Influenza A viruses cause epidemics and pandemics in humans. Being highly pleomorphic, lab-adapted strains produce spherical and bacilliform virions, while clinical isolates produce extremely long filaments. Influenza filaments are poorly characterised owing to their diverse sizes and fragile structures. Using an integrative approach combining structural and compositional studies we analysed virions in detail and show that filaments encompass distinctive features that are lacking in spherical virions.

Using mass spectrometry, we investigated the composition of purified filamentous and spherical virions. Although filaments and spheres had similar glycomes, viral filaments contained fewer classes of lipids implying their involvement in membrane curvature and budding. Comparing proteomes of both morphologies indicated a depletion of neuraminidase and an increase in hemagglutinin in filamentous virions. Cryo-electron tomography of budding filaments showed unique protein features such as fibril-like density inside virions that we structurally elucidated to be cofilin bound to cytoskeletal actin. Western blots indicated higher levels of active dephosphorylated cofilin suggesting that influenza filaments finetune and regulate cofilin expression and their depolymerising activity during infection and virus budding. Combining compositional and structural data we then produced a pseudoatomic model of an Influenza filament.

Integrating data from several sources we have produced a detailed model that describes the molecular anatomy of Influenza viral filaments. Our study shows that clinically favoured filamentous virions are structurally and compositionally different from lab-based spherical virions that are more widely used by influenza researchers. Our integrative structural model provides a basis for hypothesis-driven studies of their assembly and function.

Evaluating changes in the spike S2' cleavage site of the avian gammacoronavirus infectious bronchitis virus on virus tropism and protease usage.

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Abstract

The *Gammacoronavirus* infectious bronchitis virus (IBV) causes an economically damaging disease in chickens. IBV is controlled by live attenuated vaccines produced by serial passage of a virulent isolate in embryonated hens' eggs. Serial passage is time consuming, dependant on the supply of eggs, unable to readily adapt to emerging strains, and attenuates the virus through an unknown molecular mechanism. Therefore, generation of rationally attenuated vaccines not reliant on embryonated eggs is required.

Most IBV strains exhibit restricted tropism *in vitro*, unable to replicate in continuous cell lines. Beaudette, an attenuated laboratory strain, replicates in Vero cells, a continuous cell line licenced for vaccine production. IBV spike (S) activation, at S1/S2 and S2' cleavage sites, is required for infection. The S2' site is important for extended tropism, but the protease cleavage is poorly understood.

As vaccination with Beaudette does not induce protective immunity we generated a recombinant IBV, M41K-BSM, based on the virulent M41 strain, containing three amino acid changes in S, Pro687Arg, Arg689Lys, and Phe692Leu, mirroring the Beaudette S2' site. *In vitro* assays demonstrated the changes conferred extended tropism to M41K-BSM without impacting replication *in ovo*. *In vivo*, chickens infected with M41K-BSM exhibited reduced clinical disease, despite replication comparable to the pathogenic M41 strain suggesting protease cleavage may be a factor in pathogenicity.

We developed and utilised cell-cell S based fusion assays to explore IBV S activating proteases. Chicken serine proteases which activate MERS and SARS-CoV-2 S, also activated IBV S. Protease activation partially mapped to the S2' cleavage site.

Mutation of ICP34.5 in HSV1 reveals divergent phenotypes across common lab strains

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Abstract

The double-stranded RNA activated protein kinase (PKR) pathway is a central antiviral pathway that responds to virus-generated dsRNA by phosphorylating the translation initiation factor eIF2a, resulting in translational arrest and greatly reduced virus yields. HSV1 is particularly adept at counteracting this pathway and encodes several proteins that block it, including ICP34.5 which directs eIF2a dephosphorylation. Despite extensive research, there are discrepancies in the literature around the effect of mutating ICP34.5. Using infection of primary human fibroblasts (HFFF) as a clinically relevant model, we have analysed three HSV1 strains – S17, Sc16 and F - widely used in research as wild-type HSV1, and ΔICP34.5 mutant strains in each of these backgrounds. While Sc16 and F mutants failed to spread in HFFFs and induced high levels of PKR phosphorylation, eIF2a phosphorylation and formation of cytoplasmic RNA stress granules that indicate translational inhibition, S17 was refractory to the loss of ICP34.5. Moreover, unlike the Sc16 and F mutants, S17 ΔICP34.5 was able to maintain late protein synthesis, suggesting differential generation of PKR-activating dsRNA or functions of virus factor(s) influencing PKR responses at late stages of infection. Our results highlight that not all HSV1 lab strains are equal, and the introduction of similar mutations across different strains has unveiled a variable phenotypic background in cells competent for antiviral responses, potentially reflecting an extensive history of passage in non-human cells. We are now aiming to test which phenotype is closer to clinical HSV1 by reconstructing this ICP34.5 deletion in a new clinical isolate of the virus.

Virus Forum: Pathogens & Antivirals

Screening FDA-Approved Drugs with Recombinant Cedar Virus as a CL-2 Surrogate to Identify Potential Nipah Virus Antivirals

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Abstract

Nipah virus (NiV) and Hendra virus (HeV) are highly pathogenic zoonotic paramyxoviruses, belonging to the Henipavirus genus, that cause severe respiratory and neurological disease in humans and livestock, with high case fatality rates and no approved antiviral therapies or vaccines. Ongoing outbreaks of NiV in Bangladesh and India underscore the urgent need for effective treatments, yet research has been somewhat restricted due to containment level-4 (CL-4) requirements. Consequently, these henipaviruses are among the WHO R&D Priority Pathogens list – a group of pathogens with potential to cause a public health emergency of international concern.

To combat the demand for henipavirus antivirals, a high-throughput screening (HTS) platform was established using recombinant Cedar virus (rCedV), a closely related, yet non-pathogenic henipavirus requiring only containment level-2 (CL-2) facilities, enabling a safer and faster approach to antiviral testing. Using this platform, we screened an FDA-approved drug library to identify candidate compounds with prospective antiviral activity towards henipaviruses, utilising a drug repurposing approach that offers a more efficient and streamlined process to antiviral discovery.

Our screening yielded ten promising compounds, including agents originally developed as anti-cancer and cardiac treatments that act as translation or proteasome inhibitors. These compounds demonstrated potent activity against rCedV, with IC50 values as low as 40nM in VeroE6 and HEK293T cell lines. Mechanistic studies are ongoing to elucidate their antiviral modes of action. These findings could contribute significantly to improved standard of care for NiV-infected patients and support the development of novel NiV therapeutics.

Use of a human airway epithelium model to assess zoonotic spillover risk of H5N1 avian influenza viruses

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Abstract

Since 2020, Highly Pathogenic Avian Influenza virus (HPAIV) H5N1 of clade 2.3.4.4b has spread to six out of seven continents and caused devastating outbreaks among wild and domestic birds. The ongoing panzootic has also been characterised by an increasing number of detections in mammalian species, including a 2024 outbreak in dairy cattle in the U.S. that has resulted in numerous human cases. This may present the opportunity for H5N1 to acquire mutations that enhance its replication and spread in humans, potentially representing a heightened pandemic risk.

We undertook studies infecting human nasal airway epithelial cultures (hNAECs) with HPAIV H5N1. This is a fully differentiated model of the human airway cultured at the air-liquid interface (ALI), offering a platform to study respiratory viruses that is more representative than continuous cell cultures. By quantifying plaque forming units (PFU) of virus recovered from hNAECs after infection, we were able to compare growth dynamics of influenza virus strains and assess zoonotic spillover risk of the recent cattle genotype. We demonstrate that the cattle isolate A/dairy_cattle/Texas/24-008749-001/2024(H5N1) grows robustly in hNAEC, reaching a peak titre of 4.85 ± 0.58 $\log_{10}$(PFU/transwell) at 48 hours post infection (average of n = 9 hNAEC donors). This is comparable to an earlier HPAIV isolate with established zoonotic spillover potential, A/turkey/Turkey/1/2005(H5N1) (3.77 ± 1.14 $\log_{10}$(PFU/transwell); average of n = 9 hNAEC donors). We also identify subsets of susceptible cells and investigate host responses to infection by performing single-cell RNA sequencing (scRNA-seq) on HPAIV-infected hNAECs.

SARS-CoV-2 entry into macrophages is via an ACE2-independent mechanism and does not result in productive infection

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Abstract

SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA virus that infects epithelial cells by binding to the human angiotensin-converting enzyme 2 (ACE2). Lung alveolar macrophages (AMs) are among the first immune cells to detect SARS-CoV-2 infection and initiate a protective immune response, however in severe COVID-19, the macrophage response is hyperinflammatory and dysregulated. Although it is well-documented that SARS-CoV-2 can enter macrophages, the specific entry route remains unclear. Additionally, there is controversy regarding ACE2 expression on macrophages and their ability to sustain SARS-CoV-2 replication. In this study, we used human induced pluripotent stem cell (iPSC)-derived macrophages as a model for tissue-resident AMs. Flow cytometry analysis revealed that neither iPSC nor monocyte-derived macrophages express ACE2. By generating hACE2+ iPSC-macrophages, we confirmed SARS-CoV-2 does not enter macrophages via ACE2. Further analysis indicated an absence of macrophage receptors capable of binding the SARS-CoV-2 receptor-binding domain or the full spike protein. Single-molecule fluorescence in situ hybridization (smFISH) and negative strand-specific qRT-PCR demonstrated that macrophages do not support SARS-CoV-2 genome replication. Overall, our findings suggest that alveolar macrophages internalise SARS-CoV-2 virions via a non-specific mechanism and the ingested virus is subsequently unable to replicate. Ongoing work investigates which interactions between SARS-CoV-2 and macrophages may trigger a pro-inflammatory response.

The role of fibroblasts in the Tumour Microenvironment of Epstein-Barr Virus positive Nasopharyngeal carcinomas

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Abstract

Epstein-Barr Virus (EBV) is a human oncogenic virus implicated in the development of various lymphoid and epithelial cancers. An example of this is EBV-positive Nasopharyngeal carcinoma (NPC), an endemic malignancy in Southeast Asia accounting for over 90% of cases.

The Tumour Microenvironment (TME) in all cancers comprises a heterogeneous population of cells, ultimately leading to cancer cell survival, growth and metastasis. Transforming Growth Factor β (TGFβ) in TME has been shown to promote aggressive phenotypes in EBV-positive NPC. Similarly, TGFβ is secreted by cancer cells to stimulate fibroblast transformation into cancer-associated fibroblasts (CAFs), which have an activated phenotype within the TME. Continuous fibroblast activation results in extracellular matrix (ECM) remodelling and the activation of signalling pathways that promote tumorigenesis. We explored the synergistic relationship between NPC and fibroblast cell growth in the TME.

Monolayer cell culture studies are widely used in drug development but are non-representative of the TME. To overcome this, 3D organoid culture using multiple cell types with an ECM provides better models of *in vivo* interactions. Organoid models of EBV-positive NPC cell lines with fibroblasts revealed increased NPC organoid formation and enhanced CAF stimulation, emphasising the significance of crosstalk between the tumour and fibroblast. Cytokine arrays were used to identify secreted soluble factors from fibroblasts and CAFs, revealing an increase in the production of VEGF-A and IGFBP-3, both implicated in tumour cell invasion and metastasis. These results, paired with RNA Sequencing data, may elucidate signalling pathways used for communication between tumour and fibroblast which drive tumour progression.

CHARACTERISING THE ROLE OF SOMATOSTATIN RECEPTOR 5 IN HPV-DRIVEN CERVICAL CANCER

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Abstract

Human papillomaviruses (HPV), non-enveloped double-stranded DNA viruses, are responsible for over 99% of cervical cancers, through persistent infections with high-risk subtypes HPV16 and -18. While our understanding of HPV-driven carcinogenesis is advancing, the pathways HPV hijacks to promote carcinogenesis are not fully understood. The Hippo signalling pathway, which regulates essential cellular processes including proliferation and survival, is one such pathway co-opted by HPV. Our research shows that HPV disrupts Hippo signalling, hyperactivating the transcriptional coactivators YAP and TAZ, to drive cellular transformation. However, the downstream targets of these coactivators are not fully elucidated.

Through RNA sequencing of TAZ knockdown HPV18+ cells, we identified somatostatin receptor 5 (SSTR5) as a significantly upregulated downstream target. Utilising a series of cell-based assays using HPV18+ HeLa, HPV16+ SiHa, and HPV-negative C33a cell lines, we characterised the role of SSTR5 on classic cancer phenotypes. Overexpression of SSTR5 suppressed cervical cancer cell proliferative capacity, implicating it as a potential tumour suppressor. Examination of a cervical cancer panel, comprising HPV16+, HPV18+, and HPV-negative samples, revealed a consistent downregulation of SSTR5 in HPV+ cervical cancer, observed both at the transcriptional and protein levels. Notably, SSTR5 expression was differentially regulated by YAP and TAZ depending on HPV-status.

Our study reveals that SSTR5 is regulated by Hippo pathway coactivators in HPV-driven cervical cancer and serves as a novel tumour suppressor in this disease context. This sheds light on the previously unknown relationship between Hippo signalling and SSTR5 expression, offering valuable insights into the molecular mechanisms governing cervical cancer pathogenesis.

Phage Therapy for Antibiotic-Resistant Pseudomonas aeruginosa: Overcoming Manufacturing Barriers in the UK

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Abstract

Phage therapy against antibiotic-resistant *Pseudomonas aeruginosa* has been used in compassionate use cases and a recent Phase I/II clinical trial. However, in the UK, use has been highly limited, in-part due to the lack of regulatory framework, the personalised approach often taken for compassionate use and the need for high-quality manufacturing. Manufacturing phages to high quality (GMP: good manufacturing practice) standards has been identified as a bottleneck to the use of phages in UK by the government.

The aim of this study was to develop a manufacturing process for phages against *P. aeruginosa*, establishing a critical quality criteria, developing reference materials to verify analysis methods to support good manufacturing practice (GMP) and align with MHRA regulations.

Three genetically and morphologically distinct phages were used to determine the impact of diversity on manufacturing processes. Each step in the manufacturing process was assessed to determine the most effective and efficient way to produce phages that are safe and using methods that can be up scaled, whilst maintaining the critical quality attributes that we outlined. Purification methods were used to remove bacterial contaminants namely endotoxin, bacterial DNA and bacterial proteins, and innovative techniques were used to monitor the quality attributes. Reference materials are in development to enable these analysis methods to be verified and used in monitoring in GMP in the future.

Production of high-quality phages within the UK requires adaptation and innovative approaches however, these necessary steps will open the gates to using phages as an alternative to antibiotics going forward.

Human ACE2 transgenic pigs are susceptible to SARS-CoV-2 and develop COVID-19-like disease.

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Abstract

Animal models that closely mimic human COVID-19 are essential for advancing our understanding of disease mechanisms and for developing effective vaccines and therapies. Pigs offer a valuable model for human disease research due to their genetic, anatomical, physiological, and immunological similarities to humans, along with being an ethically favourable alternative to non-human primates. However, the natural resistance of pigs to SARS-CoV-2 infection has limited their use in COVID-19 research. To overcome this, we developed transgenic pigs that express the human ACE2 receptor, rendering them susceptible to SARS-CoV-2 infection. Upon viral challenge, these transgenic pigs exhibited clinical signs of COVID-19, including fever, cough, and respiratory distress. Viral replication was observed in the nasal turbinates, trachea, and lungs for up to seven days post-infection, and lung tissue analysis revealed immunopathological changes similar to those seen in severe human cases of COVID-19. This model establishes human ACE2-expressing transgenic pigs as a robust large-animal model that recapitulates key aspects of COVID-19, providing a critical tool for the development and testing of novel vaccines and therapeutic interventions.

Characterisation of Lactobacillus plantarum as a therapeutic and preventative strategy for RSV-disease.

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Abstract

Respiratory syncytial virus (RSV) is a significant threat to global health, affecting infants, the elderly, and immunocompromised individuals. Although recent advances in RSV research have led to the approval of vaccines to pregnant women and older adults, prevention, and treatment options to provide protection for all vulnerable populations are still needed. One promising novel approach is the use of *Lactobacillus plantarum* (LP), a probiotic species with prominent immune-modulatory properties that could potentially protect against RSV without causing excessive inflammation. Our study has evaluated the immunomodulatory effect of three LP strains against RSV in various respiratory epithelial cell models, as well as well-differentiated primary epithelial cells. Flow cytometry and confocal microscopy confirmed that LP strains interact with, and are internalized by, respiratory epithelial cells, exhibiting a strain- and dose-dependent relationship. Furthermore, we observed that LP reduces the production of interferon lambda (IFN-λ) and interleukin-6 (IL-6) in RSV-infected cells, suggesting a probiotic anti-inflammatory effect. This potentially beneficial response was only recorded following RSV infection. If the cells are first exposed to LP and then infected with RSV no significant effects were observed. Our results highlight the potential of LP as therapeutic agent, capable of preventing exacerbated inflammation. Future research examining the molecular pathways of LP-induced immune responses could further support its role as a safe, novel strategy to mitigate RSV morbidity.

MPXV Infects Human PBMCs in a Type I Interferon-Sensitive Manner

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Abstract

MPOX virus (MPXV) caused a global outbreak starting in May 2022, primarily driven by Clade IIb. Meanwhile, Clade I viruses in the Democratic Republic of Congo (DRC), including the newly identified Clade Ib, raise concerns due to increased human-to-human transmission and their continuous spread. While most infections present as mild, some can progress to severe systemic disease, highlighting the need to understand viral intra-host dissemination. To investigate the susceptibility of immune cells to MPXV, we exposed isolated human peripheral blood mononuclear cells (PBMCs) from healthy donors to a Clade IIb MPXV isolate ex vivo, in the presence and absence of IFN-α2a pretreatment. qPCR revealed increasing MPXV DNA in PBMCs over 5–6 days, indicating susceptibility to infection. Notably, IFN-α2a pretreatment reduced viral DNA levels, suggesting MPXV is sensitive to type I interferons. Plaque assays confirmed de novo infectious MPXV production in PBMCs. Single-cell RNA sequencing identified monocytes, cycling NK cells, and regulatory CD4+ T-cells as MPXV-susceptible cell types within the human PBMC population. Additionally, MPXV infection led to downregulation of innate immunity pathways, a hallmark of poxvirus infection. Treatment with antivirals Cidofovir and Tecovirimat reduced viral replication and infectivity, suggesting their potential for controlling viral spread in patients. Together, our data suggest that human PBMCs are productively infected by MPXV which is accompanied by significant modulation of the cellular milieu. Our results have the potential to illuminate aspects of intra-host propagation of MPXV that may involve a lymphohematogenous route for replication and/or intra-host dissemination.

Harnessing the antiviral potential of *Nigella sativa* volatile compounds against enveloped viruses.

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Abstract

Recent viral pandemics and outbreaks have highlighted the need for broad-spectrum virucidals that can be deployed rapidly in response to emerging viruses. Here, we investigated the virucidal activity of bioactive compounds derived from *Nigella sativa* oil, against seasonal coronaviruses, and SARS-CoV and SARS-CoV-2 pseudoviruses.

To assess the activity of the volatile components of *Nigella sativa* oils or its bioactive compounds, diffusion assays were established, where viruses were pre-treated with the active constituents by incubating in adjacent wells of microwell plates. Infectivity of the treated viruses were then determined. Pre-treatment of 16-24 hours showed significant reduction in viral infectivity (≥3 $\log_{10}$ reduction), accompanied by no effect on cell viability.

We identified four key bioactive compounds present in the oil vapour phase by mass spectroscopy, with thymoquinone showing the most potent activity. When used in combination with the other three bioactives identified, we observed a synergistic effect; lower concentrations of thymoquinone were required for a significant reduction in viral infectivity.

Haemolysis and RNA protection assays indicated that the four-compound formulation had a virucidal effect on enveloped viruses by disruption of the lipid envelope, which we also visualised by transmission electron microscopy. These data were consistent with our observations that the formulation has no impact on non-enveloped respiratory viruses.

Further experiments to delineate the contribution of each bioactive compound to the overall synergistic effect are ongoing. This is a key step towards developing broad-spectrum virucidals against respiratory viruses, which could be delivered directly to the entry site through sprays or inhalers.

Virus Forum: RNA in Viral Replication

The segment specific impact of CpG-enrichment on influenza A virus fitness

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Abstract

The genomes of all organisms display biases in their nucleotide content. For example, the dinucleotide CpG is dramatically underrepresented in vertebrate genomes. Interestingly, this repression is mimicked by the RNA viruses that infect vertebrates, including influenza A virus (IAV), a pathogen of global importance. This mimicry is thought to have been driven by evolutionary pressure to escape recognition by host cell factors including zinc-finger antiviral protein (ZAP), a cytoplasmic sensor specific for ssRNA CpG detection. Previous work characterising CpG-enrichment in two of the genome segments has illustrated that there is dramatic segment-specific variability in the impact of CpG-enrichment on viral fitness. Here we have expanded on this to produce CpG-enriched IAV segments 2, 3, 4 and 6. The greatest attenuation results from CpG-enrichment of the segments 1, 2 and 3 (encoding the PB2, PB1 and PA subunits of the viral polymerase, respectively), whilst CpG-enrichment of the other segments such as 5 (encoding the viral nucleoprotein, NP) and 6 (encoding neuraminidase, NA) is well tolerated. Viruses with CpG-enriched polymerase-encoding segments exhibit an attenuated phenotype in human lung cells and viral fitness is restored in ZAP knockout cells, demonstrating that this attenuation is ZAP-dependent. This work suggests that the polymerase segments of IAV may be the best targets for CpG-enrichment in live attenuated vaccine design in the future and highlights the potential of this approach. We are currently testing combinations of multiple CpG-enriched segments in one virus to further optimise the parameters for the implication of CpG-enrichment in live attenuated vaccine design.

Direct expression from incoming retroviral RNA genomes

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Abstract

All +ssRNA viruses translate their genomes after cellular entry to synthesize viral proteins, except retroviruses (e.g. HIV-1), which use their genomes as a template for reverse transcription. As the viral RNA in particles is almost identical to the viral RNA produced after integration, we asked whether incoming retroviral genomes could be directly translated. Using complementary methods including SILAC-based mass spectrometry, immunoprecipitation, polysome fractionation and reporter assays, we demonstrate that incoming HIV-1 genomes are directly translated after cellular entry, even in the absence of reverse transcription. This process occurs in different cell types, with different viral genomes and in the presence of diverse envelope glycoproteins. Mutations that alter capsid stability also impact direct translation by altering ribosome accessibility, whereas packaging signal mutations that prevent the incorporation of the viral genome into particles abolish this process. Viruses produced in the absence of protease activity lose the ability to translate genomes, indicating that particle maturation is required. Host factors that inhibit translation of specific RNAs also inhibit direct translation from the incoming viral genome. Using capsid mutants that are deficient in host cofactor interactions, integrase mutants that result in eccentric particles, we further probe the mechanistic details of individual steps after virus entry that are relevant to the process of incoming genome translation. Our findings challenge the notion that retroviruses require reverse transcription to produce viral proteins, and identify key viral and cellular determinants that contribute to this process.

Transcriptomic analysis of human airway cultures singly- and co-infected with SARS-CoV-2, Influenza A Virus or Respiratory Syncytial Virus

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Abstract

Previous studies have shown that in viral co-infections, SARS-CoV-2 replication can be reduced or inhibited by the antiviral response triggered by co-infecting viruses, such as rhinovirus, influenza A virus (IAV), or respiratory syncytial virus (RSV). This outcome is dependent on the timing and sequence of the virus infections. We wanted to investigate how the responses of infected human airway cultures differed in inhibitory or non-inhibitory conditions to SARS-CoV-2 in the presence of either IAV or RSV. To this end, air-liquid interface (ALI) cultures of human bronchial epithelium were either mock, singly-, or co-infected with these viruses. Single infections, as well as simultaneous and sequential coinfections were carried-out and ALI cultures were harvested for transcriptomic analysis at various times post-infection. SARS-CoV-2, IAV, and RSV, exhibited distinct transcriptomic profiles, with IAV and RSV inducing earlier and more robust antiviral responses compared to SARS-CoV-2. We identified a subset of differentially expressed genes uniquely expressed in conditions that do not support SARS-CoV-2 replication, suggesting an anti-SARS-CoV-2 role for these genes. We also investigate how co-infected tissues differ from singly-infected tissues. In sum, our results provide new information on the cellular processes that influence virus-virus interactions, shedding light on the community structure and ecology of viruses.

Characterisation of the s2m RNA structure in the replication and pathogenicity of Avian Coronavirus'.

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Abstract

The s2m, a hairpin stem loop RNA structure, is present in the 3' untranslated region (UTR) of a range of positive sense single stranded virus families, including *Coronaviridae*, *Astroviridae*, *Picornaviridae*, and *Caliciviridae*. Deletion of the s2m in SARS-CoV-2, a *Betacoronavirus*, does not affect viral replication or pathogenicity *in vitro* or *in vivo*. Surprisingly deletion of the s2m in the IBV Beaudette strain resulted in a 36 nucleotide (nt) insertion in place of the s2m, along with an upstream single point mutation.

Using reverse genetics, we altered the canonical structure and/or recapitulated the observed 36nt insertion in two strains of IBV, representing different genotypes and disease severity. We assessed the impact of these changes on RNA structure, viral replication, and disease progression *in vivo*. Selective 2'-Hydroxyl Acylation analysed by Primer Extension (SHAPE) was utilised to characterise the s2m altered variants and assess the effect of s2m disruption on global 3' UTR stability. Alterations to the s2m in different IBV strains show no difference in replication *in vitro*, however in *ex vivo* tracheal organ cultures (TOCs) one exhibited reduced replication. Alterations to the s2m, including replacement with a 36nt insertion, lead to altered phenotypes *in vivo* which were dependent on strain; this included delayed establishment of clinical presentation, reduced viral load in secondary sites of infection, and a decrease in antibody response. Understanding the role of the s2m in replication and pathogenicity of IBV may provide an important insight into its viability as a target for rational attenuation in vaccine design.

Dynamics of SARS-CoV-2 RNA-dependent RNA polymerase association with the viral genome

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Abstract

The RNA-dependent RNA polymerase (RdRp) of *nidovirales* is a molecular acrobat, coordinating both genome replication and transcription of subgenomic RNA. Together with its replicase complex, it is responsible for carrying out programmed long range RNA:RNA recombination between transcription regulatory sites (TRSes), and distinct RNA molecules, driving genetic diversity.

Since establishing in humans, novel SARS-CoV-2 variants have arisen from both mutations and recombination driven by its RdRp; understanding the dynamics of viral transcription and replication is essential to comprehend viral evolution at the molecular level.

Here, we report on RdRp dynamics during SARS-CoV-2 infection in cultured cells using individual-nucleotide resolution crosslinking and immunoprecipitation (iCLIP), which generates a snapshot of nsp12, the SARS-CoV-2 RdRp, along the viral genome. We observe consistent patterns of dynamic RdRp association during mid- (6 h.p.i.) and late- (10 h.p.i.) infection.

Our data revealed striking patterns of nsp12 association with the viral genome, identifying the putative promoter for genome replication in SARS-CoV-2 in the 3'UTR, as well as the conformation of key RNA structures to initiate transcription and replication.

Additionally, within the 5'UTR, we find that nsp12 associates with RNA structures adjacent to the TRS-Leader (TRS-L). The pattern suggests the mechanism of subgenomic RNA might require coordination between multiple RdRps, or that specific RNA structures are responsible for "capturing" the RdRp adjacent to the TRS-L.

This work offers key insights into the behaviour of coronavirus RdRp. Ultimately, the generated knowledge will advance the understanding of fundamental and conserved mechanisms regulating viral replication and driving evolution.

Elucidating Influenza A packaging interactions in the context of infection

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Abstract

The segmented nature of the influenza A virus (IAV) genome provides it with an evolutionary advantage, allowing for reassortment of virus segments upon co-infection of differing strains. However, it also poses a problem: the virus must be able to ensure that one of each of its eight segments are bundled and packaged to produce an infectious virion. These segments are organised as viral ribonucleoprotein complexes (vRNPs), which consist of viral RNA (vRNA) bound non-uniformly to nucleoprotein (NP), allowing for the formation of RNA secondary structures. Previous work from our lab has shown that these structures can facilitate vRNA-vRNA interactions within virions (*in virio*). Alongside other reports showing co-localisation of vRNPs post nuclear export, this work suggest a potential role for vRNP-vRNP interactions in segment bundling.

Here, we report on work to elucidate the IAV genome assembly pathway during infection using biochemical methods, structural sequencing techniques, and RNA fluorescent *in situ* hybridisation (FISH). We employ sequencing of psoralen-cross-linked, ligated, and selected hybrids (SPLASH) and kethoxal-assisted RNA-RNA interaction sequencing (KARR-seq) to probe long-range vRNA-vRNA interactions both *in cellulo* and within purified virions. Through comparison of these data, *in cellulo* vs *in virio* interactions, we aim to identify key inter-segment interactions that drive the IAV genome bundling process from nuclear export to virion budding. By understanding the dynamics of inter-segment interactions during IAV genome bundling, we hope to predict the likelihood of IAV strain reassortment and provide insight into the processes that underpin pandemic strain emergence.

HSV-1 VHS endoribonuclease mediates host mRNA degradation through an XRN1-independent mechanism

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Abstract

Manipulation of host RNA by virus-encoded endoribonucleases is a key regulation mechanism employed by many viruses. VHS, an endoribonuclease expressed by HSV-1 and other alphaherpesviruses, is thought to cleave mRNA near the 5' cap, allowing subsequent RNA degradation by the host exoribonuclease XRN1. This results in the global depletion of mRNA, favouring viral over host translation. Furthermore, polyA tail binding protein (PABPC1), which ordinarily shuttles between the nucleus and cytoplasm on mRNA, is relocalised to the nucleus.

Here, we have used RNAseq to determine the relative susceptibility of the cytoplasmic transcriptome to VHS and have found that this is similar when VHS is expressed during infection or ectopically. VHS was also shown to induce the dispersal of cytoplasmic P-bodies, storage sites for mRNA decay enzymes, as indicated by the redistribution of XRN1 and the essential P-body component, DDX6. Although VHS-dependent P-body dispersal was blocked in the absence of XRN1, single-cell puromycin labelling revealed that translation was still stalled, with RNAseq confirming that this was a consequence of VHS maintaining its ability to degrade mRNA in the absence of XRN1. Surprisingly, and despite ongoing XRN1-independent VHS activity, PABPC1 did not translocate to the nucleus but instead localised to novel, poly(A)+ RNA granules located beside P-bodies, suggesting that RNA degradation and clearance of poly(A) tails are separable events.

These findings provide evidence that HSV-1-induced RNA degradation may utilize a distinct mechanism from that previously reported and highlight VHS as a novel tool for understanding the mRNA decay in virus infection.

Spatiotemporal single-cell RNA sequencing of chicken trachea reveals differences in host immune responses against pathogenic and attenuated infectious bronchitis virus (IBV).

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Abstract

Infectious Bronchitis Virus (IBV), an avian coronavirus, poses a major threat to the global poultry industry. Live-attenuated vaccines (LAVs) are currently the primary defence against IBV infections, but these vaccines, produced by serial passage of a virulent field isolate in embryonated hen eggs, have been shown to potentially revert to virulence, leading to outbreaks. However, the mechanisms behind this reversion remain poorly understood.

To investigate this phenomenon, we employed single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST) to profile cell types and their spatial organisation within both healthy and IBV-infected chicken trachea. A pathogenic strain of IBV, M41-CK was passaged over 100 times *in ovo*, resulting in an attenuated strain, M41-SK. Tracheal samples were collected at days 1, 4, and 14 post-infection from chickens infected with either the pathogenic (M41-CK) or attenuated (M41-SK) virus, with a mock-infected group as a control.

Our analysis revealed distinct transcriptomic profiles that were dependent on the viral phenotype (pathogenic vs. attenuated). Using integrated computational analysis, we identified key host transcriptomic markers linked to IBV pathogenicity and localised the IBV genome to specific cell types within the infected trachea. Immunofluorescent staining further confirmed the colocalization of IBV nucleocapsid and spike proteins in these cell populations.

This study provides novel insights into the immune and pathogenic mechanisms of avian coronavirus, the evolutionary dynamics of LAVs post-vaccination, and offers valuable information to enhance the rational design of more effective IBV vaccines.

Investigating the role of the non-structural protein NSs of Oropouche virus in modulating RNA polymerase II function

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Abstract

Bunyaviruses are segmented, single-stranded, negative or ambisense RNA viruses, including important human, animal and plant pathogens. Bunyaviruses are primarily transmitted by arthropod vectors, which are becoming more widespread due to climate change, increasing the likelihood of zoonotic outbreaks. Despite several bunyaviruses causing severe or fatal disease, bunyavirus-host interactions remain poorly characterised, and few effective antivirals or vaccines exist. Reflecting this, the World Health Organisation identified three bunyaviruses as priority pathogens for research and development efforts. The bunyavirus Oropouche virus (OROV) frequently circulates in Central and South America, mainly within the Amazonian regions, and causes Oropouche fever, a mild febrile illness which can progress to encephalitis, meningitis, and death. Concerningly, there is a current outbreak with reports of infection-associated miscarriage and possible microcephaly. >500,000 cases of OROV infection have been reported, but this is likely an underestimate as several co-circulating viruses cause similar symptoms. No antivirals or vaccines against OROV exist, highlighting an urgent need to further understand its lifecycle. We conducted a whole cell temporal proteomics screen which revealed downregulation of RNA polymerase II (RNAPII) during infection. The non-structural protein NSs of several bunyaviruses inhibits the interferon response by targeting RNAPII activity, although the mechanism varies between bunyaviruses and remains unknown for OROV. We have now demonstrated OROV-NSs causes a potent dose-dependent inhibition of expression from RNAPII-dependent mammalian promoters and have identified specific residues within NSs that are crucial for this activity. These data further our understanding of OROV-host cell interactions, which could aid design of live-attenuated vaccines against OROV.

Regulation of polyadenylation in coronavirus transcripts

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Abstract

Coronaviruses have a positive-sense single-stranded RNA genome, with a conserved hexamer in the 3' untranslated region that resembles a host eukaryotic polyadenylation signal (PAS). While the viral RNA remains in the cytoplasm during infection, variation of its poly(A) tail length has been reported, suggesting potential involvement of host polyadenylation machinery in the generation of the coronavirus poly(A) tail. Using long read sequencing methods, we find that the length of poly(A) tail varies between viral sub-genomic RNAs, and it changes over time during early infection. Cis-regulation of viral poly(A) tail length is investigated using reverse genetics viruses with wild-type or mutated PAS-like hexamer. We compare the virus growth kinetics between these viruses with different starting poly(A) tail length of 33, 50, and 70. Potential host factors are screened with siRNAs. Cell lines with the auxin-inducible degron system are generated to further investigate the roles of host polyadenylation complex during infection. As the poly(A) tail is a common feature in multiple positive-sense RNA virus families, its regulation may shed light in the viral replication cycle in general.

Regulatory role of the influenza A virus NEP in viral RNA synthesis

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Abstract

Influenza A virus contains a segmented negative-sense RNA genome that is transcribed and replicated by the viral RNA polymerase (FluPol). The viral nuclear export protein (NEP) plays a role in regulating viral RNA synthesis and facilitating the nuclear export of the viral RNA genome. However, the mechanisms underlying these functions of NEP remain unclear. Here, we present the structure of NEP in complex with FluPol of the 1918 pandemic influenza A virus. The structure reveals that NEP interacts with FluPol via the N-terminus of PB1 and the C-terminal domain of PA. This interaction was validated in cells using a split-luciferase assay, where mutations in NEP at the binding interface disrupted its interaction with FluPol. Furthermore, vRNP reconstitution assays showed that NEP exerts a dose-dependent regulatory effect on viral RNA synthesis. Low doses of NEP stimulated replication, while high doses suppressed both replication and transcription. A mutant NEP that fails to bind FluPol lost its ability to inhibit viral RNA synthesis at high doses but retained its ability to stimulate replication, suggesting that NEP's stimulatory effect on replication is independent of its interaction with FluPol. The FluPol binding site for NEP overlaps with the binding sites for the C-terminal domain of host RNA polymerase II and ANP32, which are essential for FluPol's transcriptional and replicative activities, respectively. This overlap may explain NEP's inhibitory effect at high doses, as it competes with these host interactions. Overall, our findings demonstrate that NEP functions as a regulatory factor for FluPol during viral RNA synthesis.

Virus Forum: Systems Virology

Identification of novel necroptosis inhibitors encoded by Human Cytomegalovirus

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Abstract

Necroptosis is a critical intrinsic defence against virus infection that directly eliminates infected cells, preventing viral spread. Human cytomegalovirus (HCMV) is an important human pathogen causing significant morbidity and mortality in immunocompromised people, and congenital infection in 1/100 pregnancies. However, to date, only two HCMV-encoded inhibitors of necroptosis has been discovered: UL36, which targets mixed lineage kinase-like protein (MLKL) for degradation, and IE1, which inhibits receptor-interacting protein kinase 3 (RIPK3) by enhancing its ubiquitination.

Here, we performed a systematic screen of HCMV recombinants deleted for blocks of HCMV 'accessory' proteins. This identified that deletion of two viral gene blocks increased necroptosis of infected cells. Screening fibroblasts stably expressing individual genes from both blocks led to our identification of four novel necroptosis inhibitors. Notably, US22, was found to repress transcription of the key necroptosis mediator RIPK3, thereby preventing phosphorylation of the terminal necroptosis mediator, MLKL. A multi-step viral growth assay showed that deletion of US22 significantly inhibited viral replication, suggesting that US22-mediated necroptosis inhibition is essential for viral replication and spread. Further characterisation of the detailed mechanism of action of US22 on RIPK3 transcription inhibition may facilitate the development of therapeutic strategies to block necroptosis inhibition by HCMV.

Porcine genome-wide CRISPR/Cas9 screen identifies host dependency factors for Influenza A virus

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Abstract

Swine influenza virus has a significant financial impact in pig breeding, while simultaneously raising alarms over catastrophic pandemics due to its zoonotic nature. As an obligate intracellular parasite, it relies on host factors for its replication. Understanding of host-pathogen interactions is required to identify novel therapeutic targets and further design disease control. High throughput phenotypic screens can be a powerful tool in achieving this with CRISPR/Cas9 allowing systematic analysis of mammalian genomes. Here we developed a genome-wide CRISPR/Cas9 knockout screen in porcine cells infected with influenza A virus (IAV), to identify novel host dependency factors. Infected cells were sorted based on the level of IAV infection, and the functional relevance of genes enriched in each population was assessed using the MAGeCK pipeline. Several genes previously identified as essential for influenza infection, including SLC35A1, WDR7 and ATP6AP1, were also identified as important for IAV replication in our screen. This demonstrated our approach was successful. In addition, multiple novel genes were identified as porcine IAV host dependency factors, several of which are expressed in the same pathway. This pathway is potentially involved in disrupting IAV replication and is currently being characterised. This study is the first genome-wide analysis of IAV-porcine interactions in a sorted screen and will provide a greater understanding of genes involved not only in IAV cell entry, but also IAV replication. This data provides the basis for further gene characterisation and comparative studies with influenza A screens in human and chicken cells.

Characterisation of a novel human cytomegalovirus restriction factor and its viral evasion

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Abstract

Human cytomegalovirus (HCMV) is a ubiquitous and clinically significant herpesvirus that causes substantial morbidity both in immunocompromised individuals and during congenital infection. The balance between the host immune response and viral evasion strategies contribute to the outcome of infection, thus new insights into these interactions may facilitate the development of novel antiviral therapeutics.

We previously developed a multiplexed proteomic screen that identified 133 host proteins degraded early during HCMV infection, which were enriched in proteins functionally important in innate and adaptive immunity. A novel, rapidly-degraded 'hit' was Neurabin-2, a multifunctional cellular protein that regulates protein phosphatase 1 and the organisation of actin cytoskeleton. The role of Neurabin-2 in viral infection is poorly understood, and its viral antagonism is uncharacterised.

Comparing three independent CRISPR knockout clones with controls, we found that Neurabin-2 potently restricted HCMV replication and spread. However, traditional approaches to identify the viral gene responsible for Neurabin-2 degradation (analysis of block deletion viruses, mass spectrometry immunoprecipitations, BioID mass spectrometry) failed. A forward genetic screen employing an HCMV ORFeome was therefore applied, revealing that HCMV UL26 targets Neurabin-2, which we have validated orthogonally. UL26 is a relatively understudied tegument protein implicated in various stages of the HCMV replication cycle. Analysis of viral-host interactions identified that UL26 interacts with several host E3 ligases, including the CTLH complex. Further analysis of these interactions and the mechanisms of Neurabin-2 restriction may lead to therapeutic strategies to restore endogenous restriction.

Exploring the Temporal Dynamics of Herpes Simplex Virus-1 Infection in iPSC-Derived Human Neurones: A Proteomic and Transcriptomic Approach to Identify Novel Host-Pathogen Interactions.

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Abstract

Herpes simplex virus (HSV)-1 is a neuroinvasive human pathogen that persists in the sensory ganglia of infected hosts and can cause severe disease when it spreads to the central nervous system. Herpes simplex encephalitis, the most common viral encephalitis in the UK, can leave patients with severe neurological sequelae despite the availability of direct-acting antivirals. Furthermore, exposure to neurotropic viruses like HSV-1 is increasingly linked with the development of Alzheimer's disease and other associated dementias. Induced pluripotent stem cell (iPSC) technology allows culture of authentic CNS human neurones *in vitro*, facilitating the study of neuronal infection and providing an experimental platform for developing new prophylaxes and antiviral therapies. We have used this technology to perform quantitative temporal proteomic studies to assess how the cellular and plasma membrane proteomes are affected during infection, identifying significant changes in proteins involved in regulating microtube morphology, ubiquitination and antiviral restriction, alongside a surprising change in abundance of a cellular transcription factor. We therefore also employed Oxford Nanopore-based RNA sequencing to reveal marked transcriptional shifts in neuronal gene expression upon infection. This comprehensive -omics analysis of HSV-1 infection in neurones has revealed further host-pathogen interactions that are unique to neuronal infection and will lead on to studies investigating how HSV-1 modulates the abundance of these host factors.

Combining dynamic residue networks and machine learning to define the mutational landscape of SARS-CoV-2 M^{pro}: new tools for improved drug design

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Abstract

Deciphering the effect of mutations in virus proteins and predicting future mutations is crucial for designing antiviral drugs with long-lasting efficacy. While understanding the impact of current mutations on proteins is feasible, predicting future mutations due to natural evolution of viruses and environmental pressures remains challenging. Using the SARS-CoV-2 main protease (M^{pro}) as a model system, we have combined molecular dynamics with graph theory-based analysis to predict which residues of M^{pro} are likely to be sensitive or tolerant to mutation (mutational 'hot' and 'cold' spots). Comparison with deep mutational scanning data confirms the power of this dynamic residue network (DRN) approach to predict mutation 'hot' and 'cold' spots in M^{pro}, with hot spot residues being highly tolerant of mutation and cold spots exhibiting mild-to-severe functional defects when mutated. Analysis of 191,878 M^{pro} sequences from the current COVID-19 pandemic shows that individual DRN metrics have some ability to predict those residues where mutations were likely to arise. However, integrating DRN metrics with additional structural and sequence-derived metrics allowed us to develop machine learning models with significantly improved prediction of residue mutation frequency. We are now combining DRN with site-directed mutagenesis, enzyme activity assays and crystallography to define how current antiviral drugs alters the dynamics and function of M^{pro}. We anticipate that our DRN approach will help guide the development of antiviral drugs with high barriers to resistance by targeting functional residues located in/near active site or at allosteric sites that are less prone to mutation.

Genome-Scale CRISPR-Cas9 Knockout Screening in Avian Cells to Identify Host Factors Essential for Influenza Virus Infection

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Abstract

IAV is a critically important pathogen in terms of human health, food security and animal welfare. Despite coordinated international surveillance and control strategies, IAV regularly causes globally significant outbreaks of disease. Genome editing has the potential to generate livestock that are resistant to IAV, reducing the burden on the farming economy and limiting the source of future pandemic strains. However, high confidence host targets that may confer resistance in livestock must first be identified. Here, we performed two genome-scale CRISPR/Cas9 knockout screens in chicken lung epithelial cells (CLEC213s) with either the IAV PR8 reference strain or the avian UDL 3:5 reassortant. Cells were sorted by FACS based on level of influenza infection and the guides enriched in the low, mid or high viral burden population were assessed using MaGECK analysis. Several guides enriched in the bottom 5% of infected cells in both screens were found to target genes involved in sialic acid biosynthesis and N-linked glycosylation. These include SLC35A1 and SLC35A2, in addition to the avian specific host dependency factor, ANP32A. This indicated our CRISPR screens were successful and capable of identifying host specific dependency factors. MOGS and MGAT1 were among the most enriched genes and these, in addition to multiple other novel hits, were validated and the characterisation is ongoing. This work represents the first genome-wide CRISPR-Cas9 screen in avian cells and has contributed to our understanding of viral mechanisms used to infect avian cells in addition to species-specific host defence factors which may be vital to host-jumping events.

Elucidating the interactome of SARS-CoV-2 proteins with interface resolution

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Abstract

The limited sizes of viral genomes make viruses heavily reliant on host molecular machineries to replicate and spread. A common mechanism to do so is through the establishment of specific and high affinity interactions between viral and cellular proteins. Hence, understanding protein-protein interactions mediated by viral polypeptides fundamentally contributes to the uncovering of the scope of cellular functions required for virus infection.

Most protein-protein studies of viral proteins employ overexpression of tagged proteins in uninfected cells. Because overexpression of viral proteins cannot recapitulate the complexity of an infected cells, it is not surprising that the overlapping between datasets examining the same viral protein is sparse at best.

Here we apply DSS crosslinking and immunoprecipitation to cells infected with fully replicative SARS-CoV-2 to identify the interactions of viral proteins with unprecedented resolution. By exploiting the identification of crosslinker bound hybrid peptides, we can not only identify the proteins that co-precipitate with the protein of interests in native conditions, but also their interfaces, allowing us to build detailed interaction networks. Moreover, intramolecular and intermolecular DSS crosslinks allow us to model complexes using AI-instructed structural analyses.

Our approach reveals direct interactors of viral proteins that can help to uncover the mechanisms used by viruses to hijack cellular resources, while also providing putative targets for host-based antiviral therapeutic approaches.

Latent human cytomegalovirus infection targets the haematopoietic master regulator GATA2.

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Abstract

Haematopoiesis lineage commitment involves stringent genetic and epigenetic regulation that is so far only partially understood. Human cytomegalovirus (HCMV) establishes latency in CD34+ haematopoietic progenitors (HPCs) but selectively persists in the myeloid lineage where it ultimately reactivates. It has been hypothesised that latent HCMV infection may promote myeloid cell commitment, therefore understanding how HCMV interacts with cellular lineage determinants during CD34+ differentiation would reveal new insight into viral-associated reprogramming of haematopoietic cell fate. To do so, we developed a model of long-term HCMV latency which allows us to track haematopoietic identity and commitment during viral infection using transcriptomic and chromatin accessibility profiles collected over three time points to define virus-associated changes in the host. The transcription factor GATA2 is a master regulator of haematopoietic stem and progenitor homeostasis and is known to promote their differentiation into an early myeloid progenitor fate, a known reservoir of latent HCMV. A number of viral latent promoters have previously been shown to be GATA2 responsive. Using this model, consistent with previous studies, we observe increased GATA2 expression in latent cells. Additionally, we now show that this is linked to accessible chromatin in GATA2-regulatory regions during long-term latency. Most strikingly, an additional regulatory link of GATA2 expression was detected during long-term infection. This suggests that the viral-targeted change to the long-term regulation of GATA2, resulting in an overall increase of GATA2, could be epigenetically imprinted. Our results add to the growing understanding of epigenetic changes associated with latent HCMV infection to manipulate haematopoietic lineage fate.

Investigation of TRS usage in alpha and gamma coronaviruses by long read direct RNA sequencing

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Abstract

Coronaviruses express their structural and accessory genes through the production of a nested set of subgenomic mRNA (sgmRNA), which are produced through the process of discontinuous transcription. This relies on the presence of two complementary sequences known as transcription regulatory sequences, one located at the 5' end of the genome (TRS-L) and the other upstream of each of the structural and accessory genes (TRS-B). Work in the *alphacoronavirus* transmissible gastroenteritis virus (TGEV) highlighted the importance of a conserved TRS sequence to produce sgmRNA with all the TRS-Bs being identical. However, In the *gammacoronavirus* infectious bronchitis virus (IBV) the TRS-B sequence is shown to differ greatly between genes. The lack of stringency in the TRS sequence in IBV may not only indicate an increased coding capacity compared to TGEV and its close relative porcine respiratory coronavirus (PRCV), but another requirement of a TRS beside its sequence.

In this study we conducted direct RNA nanopore sequencing on chicken kidney or swine teste cells infected with IBV (M41-CK or D388 strains) or PRCV 135. The samples were run on the MinION for 72 hours. Our first aim was to identify any unknown sgmRNA produced during infection, the second aim was to elucidate the requirements for TRS usage in IBV. We found that the abundance of reads was directly influenced by the proximity to the 3' end with that read being the most abundant, although there were exceptions. There was also an observed flexibility in TRS usage for IBV as well as PRCV.

Identification of the Cellular Interactome of Seasonal and Highly Pathogenic Human Coronavirus Nucleocapsid Proteins

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Abstract

Human coronaviruses (HCoVs) can be divided into seasonal coronaviruses and those that cause severe disease. The coronavirus nucleocapsid (N) protein has a vital role in viral biology, including replication and genome packing, among others. Additionally, N can interact with host proteins, some of which may be common across coronavirus species. However, some may be unique and contribute to novel aspects of pathogenesis. The interactome of seasonal HCoVs is not well understood therefore the aim of this study is to compare the interactomes of different HCoVs. Furthermore, this research into this may help identify new therapeutics targets for existing and possible future coronaviruses.

Seasonal coronavirus and SARS-CoV-2 N proteins were cloned, tagged to eGFP and expressed in 293T cells. Expression was confirmed by immunofluorescence microscopy and western blot. Interactome studies were performed by co-immunoprecipitation purification using GFP-Trap, shotgun data-dependent acquisition mass spectrometry (DDA MS/MS) and protein identification by MaxQuant. Identified host proteins that met quality thresholds were validated using western blot and reverse pulldowns.

MS data revealed that over 100 host protein interactors (44% of all proteins identified) were common to all tested coronavirus GFP-tagged N proteins. Gene ontology enrichment analysis revealed that these host interactors consisted primarily of ribosomal proteins and RNA helicase proteins, among others. HCoV-229E N had the most interactors identified, with 212, of which 36 were unique. These shared and unique interactors are being investigated using functional assays, such as siRNA knockdowns, to assess the role of these proteins on viral biology.

Single-cell analysis of the effects of cellular dormancy on the efficacy of bacteriophages

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Abstract

Bacteriophages, viruses that prey on bacterial cells, are important regulators of microbial ecosystems in nature and have profound potential as biocontrol agents. However, as obligate parasites their effectiveness is strictly dependent on the metabolic activity of their target cells. In nature, bacterial cells often exist in dormant states and in clusters of densely packed cells, which could limit phage access and activity. Here, we use microfluidics and single-cell measurement of phage activity to investigate how the density and dormancy of cells affect the efficacy of phages.

We find that in deep-dormant *E. coli* cells, the model phage T7 enters the mode of pseudolysogeny where the host is refractory to lysis but not infection. However, upon resuscitation of the host cells, T7 proceeds to replicate and successfully lyse them.

By imaging varying cell densities of *E. coli* using microfluidic chambers with specified loading capacity, we have also found that dormant E. coli exit the stationary phase earlier when present at high cell densities. This observation indicates possible quorum sensing between *E. coli* cells enabling them to exit dormancy earlier under the same nutrient concentrations, when present in high cell densities.

This finding has significant implications in treating antibiotic-persistent bacterial biofilms or wounds through phage-treatment where bacteriophages can be employed in combination with antibiotics to eradicate antibiotic-resistant bacteria and combat hard-to-treat chronic infections.

Virus Forum: The Virosphere

Characterizing Retroviral Integrations and Their Impact on Koala Populations: Implications for Health and Conservation

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Abstract

The koala genome has been invaded by retroviruses frequently over its history. The Koala Retrovirus (KoRV) is a recent example, still capable of both vertical and horizontal transmission among koalas. It likely began integrating into the koala germline several hundred thousand years ago and shows limited presence in some populations. This ongoing integration has been linked to high cancer rates in captive koalas. Additionally, two endogenous betaretroviruses, phaCin-β and phaCin-β-like, have also colonized the koala genome. PhaCin-β invaded the germline around two million years ago, while phaCin-β-like did so approximately seven million years ago. We characterized these integrations within the koala population at the San Diego Zoo Wildlife Alliance and in several European zoos, utilizing complete pedigree data to explore evolutionary dynamics across generations. Whole-genome sequencing revealed over two thousand integration sites. These included contributions from KoRV, phaCin-β, and phaCin-β-like, observing variation between deceased and living animals. Some integrations were entirely lost from current populations, and new integrations observed in offspring were absent in parents. A significant portion of integrations shared overlap with wild koalas, notably from Queensland and New South Wales, highlighting the influence of these regions on establishing the zoo population. We identified candidate markers for health-related traits, such as cancer and fertility, useful for captive breeding strategies. Our findings aim to enhance management by selecting against specific endogenous retroviral integrations that could pose health risks.

Discovering patterns in the genome of RNA viruses that drive evolution and regulate replication

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Abstract

The distribution of nucleotides across the genomes of different viruses is non-random. The UpA dinucleotide, defined as uracil bound to adenine by a phosphate bridge, is under-represented in RNA viruses including Influenza A virus (IAV). This UpA suppression potentially aids IAV evasion of host immune responses, including 3' UpA cleavage by cellular ribonuclease latent (RNAseL) enzyme. Artificially adding UpAs to the IAV genome via synonymous mutations may reduce virus fitness while preserving wild-type (WT) epitopes. Initial findings confirm that compared to WT and control viruses, IAV mutants with UpA-high (UpAH) sequences encoding the viral PB2 polymerase or nucleoprotein (NP) exhibit impaired replication in embryonated chicken eggs and human lung epithelial cells. These mutant viruses induce type I interferon production in cells likely activating the RNAseL pathway. A comparison of viral gene expression in human embryonic kidney cells and cell-free systems reveals that UpAH transcripts encoding PB2 and NP are not synthesised to detectable levels in cells, whereas they are produced to WT levels in the absence of cellular factors, supporting the presence of cellular UpA sensors which most likely cause poor transcription or degradation of RNA. The replication of UpAH viruses in RNAseL-deficient cells does not recover to WT levels suggesting further UpA sensors are present which we are currently working to identify. This project aims to improve our understanding of UpA attenuation in IAV infections, with potential applications to other RNA viruses. Identifying factors influencing virus immunopathogenesis is crucial for outbreak preparedness and developing new antiviral therapeutics or vaccines.

An increasing threat: sandfly bites enhance susceptibility to an arthropod-borne bunyavirales infection in a mammalian host

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Abstract

Arbovirus infections have increasingly become a profound burden on public health. Inflammatory response to mosquito bites and the deposit of saliva aids in replicating arboviruses. Here, we developed an in vivo model simulating natural arbovirus transmission by sandfly vectors to investigate how sandfly saliva might increase host susceptibility to Toscana virus (TOSV), one of the causes of human infectious neurological disease and is expanding its geographical range. We established specific flow cytometry panels and genetically modified TOSV to interrogate the cellular tropism of TOSV and how sandfly saliva/bites alter it at the skin inoculation site. The quantity of virus RNA was measured by RT-qPCR, and the infectious virus was assayed by plaque assay. Our results show a statistically significant increase in viral RNA in mouse tissues (e.g., skin) when TOSV is co-inoculated with sandfly saliva, correlating with more clinical signs, including neurological symptoms and joint inflammation, than inoculation with TOSV alone. We found the virus infection by sandfly saliva/bite was more inflammatory and increased cytokine expression. We identified dermal fibroblasts as crucial for TOSV replication and our flow cytometry analysis suggested that sandfly saliva/bites alter fibroblast biology, promoting a more primitive state. We suggest that while this is an aspect of normal wound healing, it simultaneously increases their capacity for TOSV replication. Importantly, salivary microbiota was not essential for TOSV infection enhancement, pointing to other salivary factors as responsible. Understanding these mechanisms may offer insights crucial for developing vaccines against arboviruses.

Investigating the evolutionary plasticity of orthomyxovirus splicing using influenza A virus segment 7 as a model system

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Abstract

Several species of orthomyxovirus utilise mRNA splicing to produce matrix protein and an ion channel/ interferon antagonist from their matrix gene segment; IAV, ICV, IDV and Thogoto virus matrix proteins share a common ancestor but their splice donor (SD) and splice acceptors (SA) located in different regions suggesting the splice sites migrated during orthomyxovirus speciation. To test SD site mobility, we took advantage of the fact that some strains of IAV produce additional spliced mRNAs 4 and 5 using alternative SD sites. Previous work indicates 2 synonymous mutations in M are needed to activate mRNA4 SD and this leads to a viable virus that expresses an ion channel with a variant ectodomain. In contrast, 6 nucleotide changes including 3 non-synonymous changes in M1 were needed to activate SD5 and although resulting segment produced a variant M1 protein, severely affected virus viability. On the other hand, we tested mobility of SA site by introducing CAG triplets within a 27-nucleotide window around these sequences and the natural SA. Of 8 sites tested, 3 produced functional splice sites and 2 gave viable viruses. Furthermore, the viable viruses expressed M2-related proteins with longer ectodomains that altered their intracellular localisation. We conclude that while there is potential flexibility over splice site location in IAV, evolution of a new coding strategy would require multiple nucleotide mutations to activate/ deactivate new/ old splice sites, many of which are likely to require non-synonymous changes in M1, further decreasing the occurrence probability.

Characterising Mutational Pressures Exerted by Nucleoside Analogues on SARS-CoV-2 Evolution.

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Abstract

Several nucleoside analogues, including molnupiravir and favipiravir, have been licensed to treat SARS-CoV-2 infection. These drugs act by inducing mutations in the viral genome during replication. Most random mutations are likely deleterious, but their accumulation can be lethal and reduce virus replication rates. However, if some patients do not eliminate their SARS-CoV-2 infection after nucleoside analogue treatment, there remains a risk of transmitting highly-mutated viruses with unpredictable outcomes.

We have demonstrated that global SARS-CoV-2 sequencing databases contain extensive evidence of molnupiravir mutagenesis. Using a systematic approach, we found that a specific class of long phylogenetic branches, distinguished by a high proportion of G-to-A mutations, is almost exclusively found in sequences after the introduction of molnupiravir treatment in countries and age groups with widespread use of the drug. In addition, we analysed UK treatment records to confirm a direct association between these high G-to-A branches and molnupiravir usage.

Subsequent in vitro serial passaging evolution studies demonstrated persistently elevated G-to-A and C-to-U mutations even after treatment was ceased, suggesting that if the initial treatment is non-lethal, it can have long-lasting effects on viral population genetics. Several persistent mutations led to amino acid substitutions within the Spike gene in regions of potential consequence for neutralising antibody recognition and proteolytic processing. Serial passaging of SARS-CoV-2 with favipiravir revealed multiple recurring mutations in the viral polymerase, indicating potential adaptations for resistance. Understanding the mechanisms underlying these genomic changes is essential for evaluating the risks associated with the broad use of mutagenic drugs on SARS-CoV-2 evolution.

Investigating the viral characteristics of virulent systemic feline caliciviruses

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Abstract

Feline calicivirus (FCV) is among the most common viruses that infect cats worldwide, with prevalence estimated to range from 10-90% depending on the population sampled. Typically, FCV infection presents with oral ulceration, fever and in some cases can also lead to clinical signs of either pneumonia or 'limping syndrome'. However, some FCV strains have been isolated from cats exhibiting virulent systemic disease (presenting with extensive mucosal and skin ulceration, oedema, and inner organ involvement), which can lead to high morbidity and mortality. Breakthrough VS-FCV infections have been recorded in vaccinated cats; therefore, there is considerable interest in developing novel therapeutics for use in the face of VS-FCV outbreaks. However, to design effective therapeutics, it is imperative that we better understand the viral characteristics that lead to virulent systemic disease.

Here, we used classical molecular virology techniques to investigate the differences between clinical isolates of FCV that were known to cause either acute respiratory or virulent systemic disease. Using *in vitro* assays, we assessed the viral replication kinetics of avirulent and virulent strains, quantifying rates of viral translation and infectious titres. Subsequently, we resolved the molecular structures of clinical isolates representative of strains associated with acute respiratory or virulent systemic disease, observing differences in capsid flexibility. These findings provide further evidence that the viral capsid protein, VP1, might play a significant role in the pathogenesis of virulent systemic disease.

Interactions of IAV and RSV during coinfection *in vitro* and formation of structures resembling hybrid viral particles

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Abstract

Coinfections, in which multiple viruses infect a single host play a critical role in influencing transmission dynamics and clinical outcomes. In an earlier study, viral interactions were examined at the cellular level and identified the formation of hybrid viral particles (HVPs) resulting from coinfection with laboratory-adapted strains of Influenza A virus (IAV) and respiratory syncytial virus (RSV). These HVPs exhibited changes in both antigenicity and receptor tropism, suggesting an important role in virus fitness. To delve into and understand the formation of HVPs further, we infected A549 cells and differentiated human bronchial epithelial cells (hBECs) with a prototype strain of RSV and a clinical isolate of IAV H3N2, the predominant influenza subtype during the last flu seasons, as well as with clinical isolates of IAV H1N1 and RSV, allowing us to make use of a more biologically relevant system. Using fluorescence imaging, we observed filamentous structures formed *in vitro* by both pairs of viruses, resembling HVPs, carrying surface glycoproteins from both IAV and RSV and a different staining profile to what it had previously been seen. We also confirmed the presence of coinfected cells with IAV H3N2 and RSV A2 in hBECs, suggesting opportunities for the formation of HVPs in a more biologically relevant system. It is notable that in both cell systems, RSV infection was reduced in the presence of IAV. These initial findings bring us closer to answering the overarching question, which is if HVPs can happen in nature and investigate their potential implications on virus pathogenesis.

Phenotypic evolution of SARS-CoV-2 spike during the COVID-19 pandemic

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Abstract

SARS-CoV-2 variants are mainly defined by mutations in their spike. It is therefore critical to understand how the evolutionary trajectories of spike affect virus phenotypes. So far, it has been challenging to comprehensively and functionally compare the many spike proteins that emerged during the pandemic in a single experimental platform. Here, we generated a panel of recombinant viruses carrying different spike proteins from 27 variants, circulating between 2020 and 2024, in the same genomic background. We then assessed several of their phenotypic traits both *in vitro* and *in vivo*. We found distinct phenotypic trajectories of spike among and between variants circulating before and after the emergence of Omicron variants. Spike of post-Omicron variants maintained enhanced tropism for the nasal epithelium and large airways but displayed over time several phenotypic traits typical of the pre-Omicron variants. Hence, spike with phenotypic features of both pre- and post-Omicron variants may continue to emerge in the future.

Investigating the evolution of seasonal human coronaviruses and their application to future evolutionary directions of SARS-CoV-2

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Abstract

Seasonal human coronaviruses (HCoVs) account for 5-30% of respiratory infections. HCoV-229E and HCoV-OC43 were first characterised in the 1960s and HCoV-NL63 and HCoV-HKU1 in the early 2000s and continue to circulate widely. Despite this, HCoV evolutionary trajectories are poorly understood. This study aimed to sequence 1127 clinical HCoV samples collected in 2012-2022 across Slovenia, using bespoke primer schemes. Sequencing data was analysed to characterise HCoV genomic change over the ten-year period with a view to predicting the future evolutionary pathway of SARS-CoV-2. Novel primer schemes were designed using publicly available sequence data from the Sequence Read Archive (NCBI) to generate amplicons of 400bp or 1000bp spanning each HCoV genome. After successful trial with clinical samples, 1127 clinical samples were Illumina sequenced using the 400bp amplicon scheme and analysed using a bespoke bioinformatic pipeline. SARS-CoV-2 publicly available sequences were analysed in parallel to compare genomic change. 202 HCoV-229E, 547 HCoV-OC43, and 378 HCoV-NL63 usable sequences were obtained. Phylogenetic analysis suggested highly divergent sequences emerged during the ten-year period per HCoV, with variation differing year on year. Single nucleotide polymorphism (SNP) increases year to year were observed which varied across HCoV and SARS-CoV-2 proteins. Novel and previously reported amino acid substitutions were characterised in the HCoVs showing continuous evolution throughout 2012-2022 in Slovenia. Similar evolutionary patterns were observed in SARS-CoV-2 compared to HCoVs regarding SNPs, which could suggest that SARS-CoV-2 will continue to evolve and diversify over time. This is important to inform future policy, therapeutics, vaccines, and non-therapeutic interventions for SARS-CoV-2.

Exploring the glycoprotein frontier: diversity in sarbecovirus spike protein functionality and implications in spillover

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Abstract

The rise in wildlife-to-human zoonotic virus transmission demonstrates the importance of identifying mechanisms driving host jumps. The coronavirus family (CoVs), particularly sarbecoviruses, exemplify this potential with their broad host adaptability and host-range. To study intra- and inter-species functional variability, we analyzed spike proteins from four SARS-CoV-2 variants (D614, B.1, BA.2, and BA.2.86), two cryptic lineages from a chronic patient's airway and gut samples, as well as one alpha derivative detected in wastewater, together with three related bat coronaviruses (sarbecoviruses RatG13, Banal20_103, and Banal20_236). Previous research has shown that the D614G mutation likely facilitated human transmission through stabilization of the S1-S2 interaction and reduction in cell surface expression of Spike; however, wider understanding of the importance of this residue in sarbecovirus biology is unknown, as well as how this phenotype might evolve during chronic SARS-CoV-2 infection. We therefore examined the sub-cellular expression of these varied spike proteins in cell lines derived from different organs. Experiments showed that BA.2 and BA.2.86 variants restored partial surface expression of spike to D614 levels, while maintaining high spike protein presence in the endoplasmic-reticulum–Golgi intermediate compartment (ERGIC) of respiratory epithelial cells (A549 and Beas-2B) but not in 293T and Vero cells. In addition, bat coronaviruses with high amino acid identity to SARS-CoV-2 also showed high levels of cell surface expression. Phenotypic spike variation across different SARS-CoV-2 variants and sarbecoviruses may indicate between-species selection pressure and an example of convergent evolution, also having an impact on the establishment of new reservoirs.

How unique an event might the bovine incursion of H5N1 Clade 2.3.4.4b B3.13 influenza A genotype virus be?

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Abstract

The incursion of B3.13 genotype of the H5N1 Clade 2.3.4.4b of highly pathogenic avian influenza A virus (IAV) into US dairy cattle is unprecedented in the era of molecular diagnosis. This raises important questions around the likelihood of this avian-to-mammal spillover event occurring elsewhere. To address this, we challenged a wide array of bovine primary and continuous cells, including from mammary and respiratory tissue, with a panel of avian, human, swine and laboratory-adapted IAV strains and measured the kinetics of infectious virus replication. Across all virus strains, udder epithelial cells were the most susceptible, followed by lower respiratory tract cells. Upper respiratory tract cells and fibroblasts from most tissues tested were generally poorer substrates for virus growth. Virus strain had a strong influence on virus replication, varying (for instance) between undetectable and 10^8 pfu/ml in highly susceptible udder cells where most IAV strains replicated well. Importantly, viruses with high-growth phenotypes included human seasonal strains, low pathogenicity avian viruses and BSL2 reassortant viruses containing internal genes from recent UK Clade 2.3.4.4b H5N1 isolates and glycoproteins of a laboratory-adapted strain. The set of BSL2 "H5N1" reassortant viruses included a B3.13 cattle isolate and although this generally grew the best of all strains tested, it was not significantly different from all other avian isolates.

We conclude that the US bovine influenza outbreak does not simply reflect a unique adaptation of the B3.13 genotype and outbreaks of mastitis in dairy cattle should therefore include regular testing for IAV.

Virus Forum: Virus:Host Interactions

Influenza A viruses induce tunnelling nanotubes by triggering cell death

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Abstract

Influenza A viruses (IAVs) spread efficiently through the respiratory tract in the form of extracellular virus particles, but can be restricted by neutralising antibodies and antiviral drugs. IAVs can avoid this restriction by transporting viral genomes directly from one cell to the next. They can do this by inducing the formation of long, thin intercellular connections known as tunnelling nanotubes (TNTs), which are capable of trafficking viral genomes. We demonstrate for the first time that tunnelling-nanotube like structures form within IAV infected lungs. We then asked how IAVs induce these structures. We found that TNT induction cannot be induced by cytokine signalling from infected to uninfected cells, but requires IAV replication within cells. IAV replication can form filamentous virions with structural similarities to TNTs, but we found that TNT induction is independent of virion morphology. We therefore looked at the intracellular responses to infection. We found that cell death pathways triggered by IAV replication induces the formation of tunnelling nanotubes, thereby establishing routes of infection spread to other cells. In this way, the virus exploits the cell death response of its host to ensure that its infection can continue to spread even within the restrictive environment of the respiratory tract.

Co-option of mitochondrial nucleic acid sensing pathways by HSV-1 UL12.5 for reactivation from latent Infection

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Abstract

Although viruses subvert innate immune pathways for their replication, there is evidence they can also co-opt anti-viral responses for their benefit. The ubiquitous human pathogen, Herpes Simplex Virus-1 (HSV-1), encodes a protein (UL12.5) that induces the release of mitochondrial nucleic acid into the cytosol, which activates immune sensing pathways and reduces productive replication in non-neuronal cells. HSV-1 establishes latency in neurons and can reactivate to cause disease. The mechanisms regulating reactivation from a latent infection are unknown. We found that UL12.5 is required for HSV-1 reactivation in neurons and acts to directly promote viral lytic gene expression during initial exit from latency. Importantly, UL12.5 is the first viral protein identified that is required for initial exit from the latency. The direct activation of innate immune sensing pathways triggered HSV-1 reactivation and compensated for a lack of UL12.5. Finally, we found that the induction of HSV-1 lytic genes during reactivation required intact RNA and DNA sensing pathways, demonstrating that HSV-1 can respond to and active antiviral nucleic acid sensing pathways to reactivate from a latent infection.

STAT5: A Potential Mediator of HCMV Reactivation in Primary Dendritic Cells

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Abstract

Human cytomegalovirus (HCMV) establishes a persistent life-long infection, which is usually asymptomatic, but poses life-threatening risks in immunocompromised patients upon reactivation from latency. HCMV establishes latency in CD34+ haematopoietic bone marrow progenitor cells, with reactivation occurring upon terminal differentiation to myeloid dendritic cells (DCs) and macrophages. In DCs, IL-6 triggers reactivation via the concomitant activation of ERK-MAPK and Src family kinases (SFKs) which promote chromatin remodelling of repressed MIEP (Major Immediate Early Promoter) to an active form, restarting lytic gene expression. SFK activity has been shown to promote MOZ histone acetyltransferase recruitment to the MIEP which is an important step for histone acetylation at the MIEP and, consequently, HCMV reactivation. However, SFKs do not bind DNA directly leading us to investigate the mechanism that translates SFK activity into MOZ recruitment to the MIEP. A phosphor-proteomic screen performed in reactivating DCs suggested the STAT5B paralog of the STAT5 family as a candidate. Here we show that STAT5 isoform expression is elevated in DCs compared to monocytes and that the transfected MIEP is responsive to STAT5 over-expression. Consistent with this, pharmacological and genetic approaches reveal that IL-6 induced reactivation in DCs is STAT5-dependent but, interestingly, PMA induced reactivation in THP1/macrophages is STAT5-independent. Overall, these data reinforce that HCMV reactivation is ligand and cell-type specific and suggest an intriguing role for JAK-independent STAT5 activity in HCMV reactivation in response to IL-6 which we hypothesise is via SFK activation.

Host cell cycle and ribosomal resources drive phage infection outcomes

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Abstract

The efficacy of bacteriophages is intrinsically linked to the physiological state of their bacterial hosts. However, identifying which physiological factors dominate and how they regulate the infection progression has been challenging. This problem stems from the inherent heterogeneity between individual cells and their dynamic physiological states. Traditionally, the field has relied on bulk interactions and measurements to infer key parameters like “average” lysis time and burst size, which obscure the dynamics of infection progression and its dependency on infected bacterial physiology.

To tackle this, we developed a microfluidic imaging assay for tracking phage infection steps in individual bacterial cells. This method enabled us to investigate how bacterial physiology shapes infection dynamics of T7 phages. Our findings uncovered that the burst size of phage infection is predominantly determined by the host’s translation capacity. Particularly, we identified the bacterial cell cycle as a critical factor influencing infection efficiency - something that cannot be captured through bulk assays. By perturbing the cell cycle with division inhibitors, we demonstrated how shifts in physiological states can influence phage infection dynamics.

Understanding the physiological factors governing phage infection progression opens many strategies for steering the infection kinetics by perturbing cell physiology. This insight helps to potentiate phages for their applications in biocontrol, including tackling antimicrobial resistant pathogens.

A complex of three HSV-1 proteins antagonises cellular antiviral mechanisms by recruiting a cellular E3 ubiquitin ligase

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Abstract

Herpes simplex virus-1 (HSV-1) is a double stranded DNA virus that is a highly prevalent human pathogen. Commonly known as the cause of cold sores, symptoms of HSV-1 infection can range widely, from asymptomatic to rare life-threatening diseases such as encephalitis. Infection is for life: HSV-1 establishes a latent infection in neurons and can periodically reactivate and cause disease throughout the host's lifetime.

A broad range of intrinsic and innate immune mechanisms are in place in cells to defend against invading pathogens. Herpesviruses such as HSV-1 have evolved multiple mechanisms to antagonise such cellular defences. For example, promyelocytic leukaemia nuclear bodies (PML-NBs) are multiprotein complexes that can silence viral DNA, activate type I and II interferons and induce apoptosis. HSV-1 encodes ICP0, an E3 ubiquitin ligase which has been known for many years to mediate proteasomal degradation of PML-NBs. We recently found that pUL55, a poorly characterised tegument protein can also cause the disruption of PML-NBs in ICP0-null viruses. Using immunoprecipitation-mass spectrometry, we show that pUL55 forms a complex with two other viral tegument proteins: pUS10 and a viral protein kinase, pUL13. Mutations preventing interactions between these viral proteins, or inhibiting pUL13 kinase activity, prevent PML-NB disruption. Furthermore, we show that, in concert, these three tegument proteins recruit a cellular E3 ubiquitin ligase and that inhibition of this E3 ubiquitin ligase prevents PML-NB disruption. Our study elucidates the function of this newly identified tripartite tegument protein complex and reveals a novel way in which herpesviruses antagonise intrinsic and innate immunity.

FBXO8, a novel E3 ubiquitin ligase that supresses inflammatory and antiviral responses

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Abstract

Respiratory virus infections (e.g. RSV, IAV and SARS-CoV-2) are a major clinical and economic burden on the NHS. Regulation of intracellular signalling is essential to activate and deactivate innate antiviral and inflammatory responses. The ubiquitin system acts as a master regulatory network controlling cellular homeostasis. Here, we conducted an RNAi screen in airway epithelial cells (A549s) to assess the role of Cullin-RING ubiquitin ligases, the largest group of ubiquitin ligases, on NF-κB activation, a major inflammatory transcription factor. siRNA-mediated depletion of FBXO8 resulted in enhanced NF-kB activity, whereas ectopic expression resulted in the reduction of NF-κB-dependent cytokine production and the lack of nuclear p65 translocation. Reporter gene assays revealed that FBXO8 blocks NF-κB signalling at the level of or downstream of TRAF-6 and upstream of the IKKs, inhibiting also the AP-1 signalling pathway. Stable depletion of FBXO8 resulted in the increased production of NF- κB-dependent cytokines, IFNβ and interferon stimulated genes in response to respiratory syncytial virus (RSV) infection and was shown to reduce the production of infectious RSV in a JAK-dependent manner. Our work opens questions about how FBXO8 is acting at the molecular level and reveals novel players in inflammation and immunity that provide novel avenues for therapeutic control of inflammatory conditions.

Vitamin D increases host resistance to arbovirus infection

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Abstract

Climate change is transforming the geographic range of arbovirus infections and increases risk of infection. Our research focuses on understanding the factors that define host susceptibility to these infections. This is crucial given the lack of effective vaccines or treatments. Infection of skin at arthropod bites is a key stage of infection. Host responses at this site significantly modulate outcome to infection. The ability of environmental and dietary factors to modulate skin response to virus are poorly defined. Here, we explored whether vitamin D, a key dietary component that has pleiotropic effects on vertebrate immunobiology, modulates the skin susceptibility to virus. Our findings show that administration of vitamin D precursor D3 into the inoculation site substantially increased host resistance to virus. Decreased virus titres were associated with significant upregulation of important antiviral innate immune genes, including interferon (IFN)-stimulated gene transcripts. In the absence of type I IFN signalling, administration of vitamin D was no longer able to provide enhanced resistance to virus. In vitro treatment of key cellular targets of virus with either D3 precursor or active, mature vitamin D, resulted in reduced virus infection, indicating that this effect was cell autonomous. Together, these results suggest that supplemental vitamin D can play a vital role in enhancing skin innate immunity to virus infections.

Human cytomegalovirus interferes with the BTK-DDX41-STING signaling axis during lytic infection

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Abstract

The innate immune response is the first line of defense against invading pathogens, including the betaherpesvirus, human cytomegalovirus (CMV). The host's innate response acts as the first line of defense, and CMV, like other viruses, has consequently evolved multiple mechanisms to manipulate host interferon (IFN) responses. DEAD-box Helicase 41 (DDX41) is an intracellular dsDNA sensor that, upon activation by Bruton's tyrosine kinase (BTK), triggers type I IFN production through the Stimulator of Interferon Genes (STING) signaling pathway. Here, we show the activation of this signaling pathway during lytic CMV infection, wherein BTK, DDX41, and STING are activated through tyrosine phosphorylation, and both DDX41 and BTK interact with STING. Further, CMV infection re-localizes DDX41 from the nucleus to the cytoplasm, where it localizes to the perinuclear virus assembly compartment (vAC). Here, DDX41 phosphorylation is attenuated, suggesting cytoplasmic redistribution leads to a less active or inactive form. Additionally, DDX41 co-localizes in the vAC with the CMV tegument proteins, pp65 and pp71, each of which interact with DDX41 in immunoprecipitation assays. We further demonstrate the protective role of this signaling pathway, as treatment with the BTK inhibitor, orelabrutinib, attenuates DDX41 phosphorylation/activation and supports increased expression of viral proteins and virus replication. In sum, our work highlights the important role of BTK-DDX41-STING signaling in the innate immune response against CMV, which the virus subverts by attenuating its cytoplasmic activity, thereby diverting it from its typically protective function.

Investigating the contribution of the AURKA-FBXL7 axis on HPV18 oncogenic development

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Abstract

Human papillomaviruses (HPVs) infection can induce modifications of host cellular chromatin and cause epigenetic changes that result in abnormal expression of cellular genes. Fbxl7 (F-box and leucine rich repeat protein 7) is a ubiquitin E3 ligase involved in ubiquitination-mediated degradation of its substrates. Aurora kinase A (AURKA) is a recognised substrate of FBXL7 while Forkhead box protein P1 (FOXP1) is a transcriptional regulator of Fbxl7. Dysregulation of FBXL7 and downstream effect on its substrate can contribute to oncogenic development in HPV18 infection. This study shows transcriptional repression and decreased protein levels of FBXL7 upon HPV18 infection and disease progression via the Aurka-FOXP1 axis.

In this study, uninfected, HPV18 infected and HPV18 integrated cells were used to model disease progression. Transcriptomic analysis of HPV18 infected cells using RNA-sequencing and Real-Time Reverse transcription-quantitative polymerase chain reaction analysis (RT-qPCR) showed decreased transcripts levels of FBXL7 and increased levels of AURKA. Western blot analysis showed that protein levels of FbxL7, Aurka and FoxP1 were altered as the disease progressed. Chromatin Immunoprecipitation (ChIP)-qPCR established localisation of FoxP1 at two different regions in the promoter of FBXL7. This suggested transcription silencing of the FBXL7 gene in the HPV18 infected cell lines by FOXP1. Enrichment of H3K27Me3 is also observed at these promoter regions, indicating epigenetic repression. AURKA inhibition by Alisertib (ALS) was not observed to restore the expression of FBXL7 in HPV18 infected cells. Overexpression of FBXL7 was also carried out to study the impact of increased FBXL7 levels on AURKA.

Deciphering the molecular mechanisms behind BstA anti-phage activity

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Abstract

Temperate phages are abundant in bacterial genomes, residing as vertically inherited islands known as prophages. As such, prophages are susceptible to phage predation on their host bacterium. The prophage BTP1, present in *Salmonella enterica* subsp. *enterica* serovar Typhimurium strain D23580, encodes a phage defence system called BstA. BstA targets exogeneous phage replication and mediates abortive infection to protect the bacterial population. However, during prophage replication, BstA is inhibited by a DNA element also encoded in BTP1 called anti-BstA (*aba*). Although, the precise molecular mechanism behind the BstA anti-phage system and self-inhibition is still unknown

To investigate BstA mechanism of action, site-directed mutagenesis was used to target highly conserved residues within the BstA sequence, two in the N-terminal domain, which contains the predicted DNA binding domain and two in the C-terminal domain, a domain of unknown function. Plaque assays demonstrated that the mutations generated ablate the defence system against exogenous phages infection. To further characterize BstA phagemids carrying the DNA replication module of the phage P22, known to infect D23580, were employed. Phagemid assays highlighted the impact of the mutations depending on the domain. Disruption to the DNA binding domain, inhibits the ability of *aba* to inhibit the defence system. Additionally, mutations in the C-terminal domain seem to affect the defence system capacity to inhibit exogenous phage replication module. Electromobility shift assays correlated the phagemids results.

These findings suggest the dual functionality of BstA highlighting a role division in the exogenous phage control and self-regulation.

IAV M2 proton channel activity targets autophagy protein LC3 to Rab11-positive endosomes.

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Abstract

The M2 proton channel of influenza A virus (IAV) causes accumulation of the autophagy protein LC3 at intracellular membranes. We have previously shown that this process is due to **C**onjugation of **A**TG8s to **s**ingle **m**embranes (CASM), a process distinct from autophagosome formation.

M2 functions to maintain the folding state of the viral Haemagglutinin (HA) protein in late and post-Golgi secretory compartments. M2-induced LC3-punctae colocalise with Rab11, indicating that these vesicles are recycling endosomes. 3D correlative light and electron microscopy (CLEM) confirmed that these vesicles are single membrane vesicles, not double membrane autophagosomes. HA travels through this LC3-positive recycling endosome compartment.

Rab11 is crucial for trafficking of the genome-containing viral Ribonucleoprotein (vRNP) complexes and the formation of viral condensates, which are thought to facilitate bundling of the eight genome segments. LC3 vesicles colocalised with viral nucleoprotein (NP) in the perinuclear region and with vRNP condensates in the cell periphery. Our data are consistent with M2 proton channel activity resulting in the conjugation of LC3 to endosomes that either are or become Rab11 positive and that - due to binding of vRNPs to Rab11 - are transported to vRNP condensates. The role of LC3 lipidation for vRNP and HA trafficking will be discussed.

Our lab has recently shown that SARS-CoV-2 E protein, also a viroporin, also induces CASM and that inhibition of this pathway results in a late stage defect in the virus replication cycle. This highlights the significance of this pathway for virus infections.

Characterising the subcellular transport of influenza A virus vRNPs and virion formation via in situ cryoET

Alasdair Hood ORCID iD, Misha Le Claire, Loic Carrique, Jonathan Grimes

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Abstract

Influenza A virus (IAV) is a seasonal zoonotic pathogen which is estimated to infect up to a billion people per year and cause hundreds of thousands of deaths. IAV has a negative sense RNA genome consisting of eight segments which are packaged into viral ribonucleoproteins (vRNPs) in association with the viral polymerase complex and nucleoprotein. During productive IAV infection, new vRNPs are generated in the nucleus and must be trafficked to the sites of virion formation at the plasma membrane, however the molecular detail of this process remains incompletely characterised.

Here we generated a strain of IAV with fluorescently tagged vRNPs to perform targeting of focused ion beam (FIB) milling of cells to allow collection of cryo-electron tomography (cryoET) data in situ in infected A549 cells. This approach has revealed substantial viral reorganisation of the ultrastructure of the cell including the generation of elongated vesicles lined with hemagglutinin (HA) and large ordered assemblies of the M1 matrix protein. It appears that the HA lined vesicles are used as a scaffold for association of vRNPs; and a direct interaction between the viral polymerase complex and the cytoplasmic tail of HA has been confirmed *in vitro*.

Furthermore, targeted FIB milling and cryoET has allowed the characterisation of virion budding at cell membranes and revealed a substantial degree of variability in the M1 matrix layer. This work has identified novel interactions between viral proteins which appear crucial for IAV vRNP transport and virion formation and may inform novel drug discovery approaches.

Virus Symposium: Impact of the climate crisis on emerging viruses

Invited: How climate change influences viral emergence by altering animal behaviour and ecology

Gregory Albery ORCID iD

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Abstract

The climate is changing rapidly, accelerating our need to understand how its effects are altering the ecology of disease on a global stage. Because the climate influences animals' behaviour across a wide range of scales, the effects of a warming world are expected to be diverse and wideranging, with important repercussions for wildlife health and animal-human interactions. Emergence of wildlife diseases into human populations is widely expected to change rapidly as a result. In this talk, I outline how climate change is influencing viral emergence via animal behaviour, drawing from a number of wild animal studies across a wide range of scales. I discuss micro-scale responses to environmental stress and acute natural disaster events, identifying how they influence socio-spatial behaviour and interactions among individuals, with complex and profound implications for viral epidemiology and spillover dynamics. Building to global scales, I move to discuss how a changing climate is altering the global mammal community via animals' habitat selection behaviours, likely bringing new competent hosts into contact with important viral reservoirs, and possibly facilitating disease emergence as a result. In doing so, I outline a few of the diverse mechanisms by which climate change is facilitating spillover of well-known and novel viruses, presenting a vital frontier for our understanding of the virome in the Anthropocene.

Mosquito saliva enhances pro-viral vascular barrier leakage through direct modulation of kinase pathways.

Liam Barningham ORCID iD

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Abstract

The Aedes aegypti mosquito is the key vector that transmits arboviruses, including those that cause dengue, chikungunya, and Zika, infecting millions annually and imposing an urgent health burden. Global warming, coupled with increased urbanisation, deforestation, and globalisation, is expanding the habitat of A. aegypti. Despite the urgent need for therapeutics, there remains a gap in treatments. Pan-viral treatments that target arbovirus transmission have much potential, although the events that occur at the vector-vertebrate interface are only now being explored.

During transmission, the vertebrate host response to mosquito saliva at the bite site enhances infection and worsens disease outcomes. Here, A. aegypti saliva increases vascular permeability, facilitating the influx of virus-permissive immune cells, which subsequently become infected and enhance viral replication. However, the molecular and cellular basis by which saliva has this affect is unknown.

Using immunofluorescence microscopy, flow cytometry, and permeability assays, we show that A. aegypti saliva significantly increases the permeability of primary human monolayers of endothelial cells, accompanied by internalisation of Vascular Endothelial Cadherin and reorganization of the actin cytoskeleton. Saliva-treated endothelial cells also exhibit increased adherence of CD14+ cells and upregulation of adhesion molecules ICAM-1 and PECAM-1. Crucially, using novel ‘Kinome’ profiling of saliva-treated endothelial cells, we identified the activation of multiple signalling pathways including MAPK and SRC that likely underlies the ability of saliva to modulate endothelial barrier function.

Together, these findings offer insights into the molecular mechanisms by which Aedes saliva enhances viral transmission and provide a foundation for the development of novel antiviral interventions.

Impact of Climate-Driven Changes on the Seasonal Distribution of Avian Influenza Virus in Ireland: Cross-Species Surveillance Imperatives

Maeve Louise Farrell ORCID iD[1], Paule-Emilie Ruy[2], Katie O'Dwyer[2], Christina Byrne[3], Ana P Vale[1], Laura Garza Cuartero[3], Gerald Barry[1]

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Abstract

Avian influenza viruses (AIV), particularly highly pathogenic avian influenza (HPAI), affects animal and human health worldwide, with rising detections across species highlighting the need for enhanced surveillance. Climate change, through its impact on temperature and seasonality, may be altering virus transmission dynamics and increasing cross-species spillover risks. This study investigates AIV prevalence and seasonal distribution in Ireland to inform biosecurity and conservation measures in a changing climate.

Data on indication of exposure to AIV in Ireland were compiled from public databases and research studies, covering avian species, red foxes and American mink across wildlife and domestic populations. Analysis in R Studio calculated the mean ($\bar{x}$), and standard deviation (SD) of seasonal AIV occurrences.

Since 2003, Ireland has recorded 3,018 AIV indications, peaking in 2019 (n = 873). Contrary to traditional expectations, most occurred in summer (n= 1,029, $\bar{x}$ 343, SD 131.4), followed by winter (n= 880, $\bar{x}$ 293.3, SD 70.2), suggesting climate-linked shifts in virus seasonality. Spring and Autumn recorded 575 ($\bar{x}$ 191.7, SD 72.8) and 534 occurrences ($\bar{x}$ 178, SD 177.7), respectively. These differences were not statistically significant ($p > 0.05$), due to high month-to-month variability.

These findings highlight that AIV incidence in Ireland is increasing during warmer periods, reflecting a potential influence of climate change on virus persistence and spread. Annual AIV surveillance across species is essential to anticipate climate-related shifts in viral ecology, strengthen biosecurity and to inform response strategies to safeguard wildlife and public health against evolving zoonotic threats.

A role for mosquito NF-κB-mediated innate immunity in restricting orthoflavivirus replication and emergence

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Abstract

One third of all emerging infectious diseases are vector-borne, with the vector's ecology and physiology playing key roles in determining whether viruses can access new host species and spread globally. Innate immunity is a known barrier to virus replication in mosquito vectors that influences vector tropism. We recently showed that double-stranded RNA and infection with model viruses activates NF-κB-mediated innate immune responses in *Aedes aegypti*-derived Aag2 cells. We have now generated novel CRISPR-Cas9-mediated knockouts in the NF-κB family transcription factor Rel2 in Aag2 cells and tested the impact on the replication of a diversity of arboviruses (flaviviruses, alphaviruses and bunyaviruses). We found that NF-κB-mediated immunity has broad antiviral activity against the *A. aegypti*-borne orthoflaviviruses dengue virus (DENV), yellow fever virus (YFV) and Zika virus (ZIKV) in mosquitoes. In contrast, little impact of NF-κB-loss-of-function was observed with the alphavirus chikungunya virus (CHIKV), indicating specificity in the role that NF-κB-mediated immunity plays in defending against infection with positive-strand RNA viruses. Using orthoflaviviruses with different transmission routes (mosquito-borne, tick-borne, no known vector), we demonstrated that NF-κB-mediated immunity is not the only molecular barrier influencing the ability of orthoflaviviruses to use *A. aegypti* as a vector. Overall, our work demonstrates the importance of mosquito NF-κB-mediated innate immunity in suppressing arbovirus replication, and shows that the barriers for arboviruses to adapt to new vector species are multifactorial and virus-specific. Our findings increase our understanding of the molecular barriers influencing arboviral emergence, and could inform the development of refractory mosquitoes incapable of transmitting human pathogens.

Virus Symposium: Into the Virosphere: exploring the full diversity of virology

Invited: Tomato brown rugose fruit virus: The other global virus pandemic…

Adrian Fox ORCID iD[1], Aimee Fowkes[1], Yue Lin Loh[1,2], Anna Skelton[1]

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Abstract

In 2014/15 a novel *Tobamovirus*, tomato brown rugose fruit virus, was discovered in Jordan and Israel. As the virus can overcome the resistance genes to other tomato infecting tobamoviruses it presents a major constraint to tomato production. The virus has rapidly spread around the globe, and now affects every continent where tomatoes are grown. Due to the damage caused to the tomato industry, there has been a worldwide effort to characterize the virus, to understand its ecology, and to try to mitigate the impact of the virus. The virus is contact transmissible and is also transmitted via seed. Once infected the virus can spread rapidly through a glasshouse by normal working procedures. The research conducted at Fera in the UK and through international collaboration has shown that the virus is robust, and can survive at least four weeks and up to six months on glasshouse surfaces. The virus is released from plants into the wider environment through pollen, dust, and irrigation water, all of which can lead to onward infections in crops. The results of ongoing research such as sequencing studies to support source tracking, and experimental work on virus release from plants and survival in growing media will be discussed in the context of the changing risk pathways presented by the seed, fruit in the retail trade, and carry over from environmental contamination.

Invited: Exploring genetic innovation throughout the evolutionary history of RNA viruses

Mary Petrone ORCID iD[1,2], Joe Grove[3], Rhys Parry[4], Julien Melade[1], Jonathon Mifsud[1], Kate Van Brussel[1], Ezequiel Marzinelli[5], Edward Holmes[1]

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Abstract

The evolution of marine invertebrates predated that of the Vertebrata by hundreds of millions of years. Thus, viruses that infect these basal animals can provide valuable insights into the long-term evolutionary history of their vertebrate-infecting counterparts. To this end, we characterised a ~40kb flavi-like virus identified in the metatranscriptome of a sea sponge. In addition to representing the longest known RNA virus outside of the order *Nidovirales*, this virus did not encode any known error-correcting mechanism. Instead, we found evidence for the capture of genetic material from bacteria, indicating that numerous strategies may be available to RNA viruses for overcoming constraints on their genome size. We observed similar innovations in RNA viruses associated with ascidians (Urochordata). Select ascidian-associated viruses in the orders *Nidovirales* and *Mononegavirales* encoded alpha-like E1 glycoproteins, likely acquired through horizontal gene transfer. Strikingly, these glycoproteins shared minimal sequence similarity to that of an ascidian alphavirus, indicative of disparate evolutionary trajectories. The fusion proteins utilised by ascidian-associated nidoviruses exhibited broader diversity with minimal structural homology to known Class II fusion proteins, and we observed a history of glycoprotein switching in this order. Taken together, we present evidence that ancient viral lineages did not evolve linearly. Our findings suggest that their history was punctuated by “genetic piracy” events, which facilitated additional diversification. The continued exploration of the marine virosphere is likely to reveal further instances of such genetic innovation.

Invited: Nucleocytoviricota viral factories are transient organelles made by liquid-liquid phase separation

Hugo Bisio ORCID iD

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Abstract

Phase separation is a widespread mechanism in viral processes, mediating replication, host manipulation and virion morphogenesis. The phylum *Nucleocytoviricota* encompasses diverse and ubiquitous viruses, including *Poxviridae,* the climate-modulating *Emiliania huxleyi* virus and other so-called Giant Viruses. Cytoplasmic members of this phylum form viral factories but their nature has remained unresolved. Here, we demonstrate that these viral factories are formed by liquid-liquid phase separation. We prove that mimivirus viral factories are formed by multilayered phase separation, orchestrated by at least two scaffold proteins. To extend these findings across the phylum *Nucleocytoviricota*, we developed a bioinformatic pipeline to predict scaffold proteins based on a conserved molecular grammar, despite major primary sequence variability. Scaffold candidates were validated in *Marseilleviridae* and *Poxviridae*, highlighting a role of H5 as a scaffold protein in the vaccinia virus. Finally, we provide a repertoire of client proteins of the nucleus-like viral factory of mimivirus and demonstrate important sub-compartmentalization of functions, including those related to the central dogma. Overall, we reveal a new mechanism for an organelle to deploy nuclear-like functions entirely based on phase separation and re-classified phylum *Nucleocytoviricota* viral factories as biomolecular condensates.

Invited: A Thirty Thousand Year Perspective on Human DNA Virus Evolution

Charlotte Houldcroft

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Abstract

As virologists, we learn that double-stranded DNA viruses are ancient and ubiquitous. But we often struggle to put accurate dates on just *how* ancient modern patterns of diversity are. Can ancient virus DNA provide a new source of data to answer these questions? We have the further conundrum that, if human dsDNA viruses are ubiquitous, why is their ancient DNA so hard to find?

Taking advantage of ancient DNA from alpha and beta herpesviruses and adenoviruses, we will explore the limits of what ancient virus DNA can currently tell us about the co-evolution of these pathogens with humans. Special guest appearance from the Palaeolithic Tooth Fairy.

Bat-specific adaptations in interferon signaling and GBP1 contribute to enhanced viral tolerance

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Abstract

Bats are reservoirs of emerging zoonotic viruses of concern that cause severe disease in humans and agricultural animals. These viruses include SARS-CoV, SARS-CoV-2, MERS-CoV, ebola and Marburg viruses, and Nipah and Hendra viruses to name a few. However, it is poorly understood how bats are able to tolerate diverse viral infections, knowledge that could help pave the way for new therapeutic strategies. Here, we characterized antiviral pathways in two divergent bat species, *Pteropus alecto* and *Eptesicus fuscus*, identifying unique bat-specific mechanisms underlying their enhanced antiviral tolerance. We demonstrate the critical roles of STAT1 and STAT2 in IFNβ signaling, along with species-specific adaptations that collectively contribute towards a "steady and ready" antiviral state in bat cells. Unlike in humans, we find that bat interferon signalling processes resist the immune antagonistic properties of viruses like MERS-CoV which further explains the ability of bats to tolerate coronavirus infections. Using transcriptomic analysis, we identified canonical and non-canonical interferon stimulated genes (ISGs) including two key bat genes, *IFIT1* and *GBP1.* Compared to their human orthologs, we show that bat IFIT1 and GBP1 exhibit enhanced antiviral activity against a wide range of RNA and DNA viruses, including coronaviruses and additional bat-derived poxviruses (e.g., Eptesipoxvirus). Ultimately, our work provides important insights into the evolution of enhanced interferon-mediated antiviral responses in bats, contributing to their unique ability to resist viral diseases.

Evidence for early and ongoing adaptation of avian H5N1 in US cattle.

Jessica Quantrill[1,2], Vidhi Dholakia[3], Jiayun Yang[2], Jack Hassard[1], Jin Yu[1], Samuel Richardson[2], Rute Pinto[4], Maryn Brown[1], Nunticha Pankaew[4], Fernando Capelastegui[3], Kolli Reddy[2], Samuel Jones[2], Mehnaz Qureshi[2], Ian Brown[2], Wendy Barclay[1], Paul Digard[4], Yan Liu[1], Stuart Haslam[1], Munir Iqbal[2], Daniel Goldhill[3], Thomas Peacock[2,1]

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Abstract

In 2020 a novel H5N1 avian influenza virus genotype emerged and, during the following years, spread around the globe triggering a panzootic. While the initial reservoir and vector for virus spread was wild waterfowl and seabirds, this virus also showed a high propensity to infect and transmit between mammalian species. This was exemplified in 2024 when H5N1 was found to be transmitting at a high level between dairy cattle in the USA, frequently spilling back over into poultry, wild birds and other mammals including humans.

Here we investigated the molecular and virological evidence that these cattle H5N1 viruses rapidly accumulated and are continuing to gain adaptations that allow them to better infect and transmit between cattle. We also investigate whether these adaptations enhance the ability these viruses to infect humans, resulting in zoonotic infections or triggering a new pandemic. In particular, we present evidence that early and ongoing adaptations have occurred in the virus polymerase and haemagglutinin genes that allow for more efficient infection of cattle and other mammals.

Viro3D: species-centred database of viral protein structures

Ulad Litvin ORCID iD[1], Spyros Lytras ORCID iD[2], Alexander Jack[1], David Robertson[1], Joe Grove ORCID iD[1], Joseph Hughes ORCID iD[1]

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Abstract

Viruses are molecular parasites infecting cellular organisms from all domains of life. Although they play crucial roles in microbial communities, they also cause severe disease in humans, domesticated animals, and plants. The necessity to escape host immune response, in combination with high mutation and recombination rates, leads to the rapid diversification of viral genes that hampers their functional annotation and phylogenetic inference. On the other hand, the tertiary structure of viral proteins tends to be more conserved and, therefore, can be used for homology searches and evolutionary analysis of deeply divergent proteins. For various reasons, structures of viral proteins are traditionally underrepresented in public databases, but recent progress in protein structure prediction allows us to address this issue. By combining two state-of-the-art computational approaches, ColabFold and ESMFold, we predicted models for 85,000 proteins from 4,400 animal viruses that expanded the structural coverage for viral proteins by 30 times compared to that of experimental structures. We also performed structural and network analyses of the models to demonstrate their utility for functional annotation and inference of deep phylogenetic relationships. To simplify the access to protein models, we created a species-centred structural database called Viro3D. It has a built-in genome browser, allows users to visualise and download protein models from a virus of interest and explore similar structures present in other virus species. This structural analysis, in combination with the Viro3D database, will benefit the virology community and facilitate virus research.

The evolutionary paradox of host-virus interactions

Tzachi Hagai ORCID iD, Gal Shuler

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Abstract

Evolutionary changes in the host-virus interactome can alter the course of infection, but the biophysical and regulatory constraints that shape interface evolution between interacting host and viral proteins remain largely unexplored. Here, we focus on viral mimicry of short host-like peptide motifs that allow binding to host domains and modulation of cellular pathways. We observe that motifs from unrelated viruses preferentially target conserved, widely expressed and highly connected host proteins, enriched with regulatory and essential functions. The interface residues within these host domains are more conserved and bind a larger number of cellular proteins than similar motif-binding domains that are not known to interact with viruses. In stark contrast, rapidly evolving viral-binding human proteins form few interactions with other cellular proteins, display high tissue specificity and their interface residues have few inter-residue contacts. Our results distinguish between highly conserved and rapidly evolving host-virus interfaces, and show how regulatory, functional and biophysical factors limit host capacity to evolve, allowing for efficient viral subversion of host machineries. These results have importnat implications for our understanding of zoonotic events where novel host-virus protein interactions may evolve and for designing new antiviral drugs trageting interafce regions between host and viral proteins.

Additional details: Shuler and Hagai, Cell Reports, 2022

Streptococcus suis as a Model for the Phage-Based Biocontrol Agents

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Abstract

(Bacterio)phages and their products are increasingly explored as alternatives/adjuncts to conventional antibiotics in controlling bacterial pathogens across the farm-to-fork continuum. However, some bacterial targets remain underexplored for phage-based controls. This is attributed to challenges in isolating phages and/or propagating them *in vitro*. One such bacterium is the zoonotic pig pathobiont *Streptococcus suis*. To date, only one virulent phage has been characterised against *S. suis*. In this study, we aimed to optimise protocols to isolate phages against *S. suis* and characterise them extensively using *in vitro* and *in silico* methods.

Pig saliva and post-mortem tissues collected from 30 farms across Ireland were screened against a panel of 50 *S. suis* strains, including the commonly reported zoonotic serotypes 2, 9 and 14. We explored UV-C and mitomycin C methods for inducing prophages from host. Through serendipitous discovery, we developed a novel temperature-dependent threonine induction method for prophage induction.

Consequently, we isolated two phages targeting the pathogen, Bonnie from pig tissue and the Clyde through induction. Sequence data was used to investigate genome composition, taxonomy, and pangenome of the phages alongside closely related phages. We identified and characterised a novel adhesion device unique to Bonnie. We propose two new single-species genera be established with Bonnie and Clyde as sole members. Host range analysis revealed the phages can infect 58/100 pathogenic strains of various serotypes and geographic origins. Furthermore, we evaluated the potential of using the phages individually or in a cocktail to control polyclonal *S. suis* infections *in vitro*.

The Good, the Bad and the Ugly: Expanding the Virosphere with Viromics

Ryan Cook ORCID iD[1], Andrea Telatin ORCID iD[1], George Bouras ORCID iD[2], Antonio Pedro Camargo ORCID iD[3], Martin Larralde ORCID iD[4], Shen-Yuan Hsieh ORCID iD[1], Fiona Newberry ORCID iD[5], Mohammad Adnan Tariq ORCID iD[6], David Baker[1], Simon Carding ORCID iD[1], Robert Edwards ORCID iD[7], Evelien Adriaenssens ORCID iD[1]

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Abstract

Viral metagenomics, or viromics, has massively expanded our knowledge of global viral diversity and shed light on the ecological roles of phages in a plethora of environments. However, the field of viromics is relatively new, with technological and methodological advancements being continually developed. Furthermore, the majority of bacteriophage diversity remains uncharacterised, and new intriguing mechanisms of their biology are being continually described. Our efforts to advance virome methodology can easily be divided into three broad categories: good, bad, and ugly.

The Good; as enigmatic gut viruses are known to re-purpose stop codons to encode amino acids, we modified versions of commonly used annotation tools, Prokka and Pharokka, for the automatic prediction of stop codon reassignment in viral genomes prior to annotation. Prediction of stop codon reassignment prior to annotation significantly improved the quality of annotations for viral genomes that use alternate translation tables.

The Bad; we investigated the presence of contaminating viral sequences in viral metagenomes, and found viral contaminants to be diverse and study-specific. We appeal for the widespread adoption of extraction controls in viromics.

The Ugly; we compared Illumina and Nanopore sequencing using different assembly strategies, including hybrid approaches, for the recovery of viral genomes from natural samples. If using one technology only, we suggest Illumina due to its superior recovery of fully resolved viral genomes and minimal erroneous genomes.

Continual optimisations of laboratory and bioinformatic workflows for viromics will help to characterise more viral diversity within samples, further elucidating the vital ecological roles of phages in nature.

Attenuation of the 2022 global monkeypox virus relative to its endemic clade IIb ancestor

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Abstract

Monkeypox virus (MPXV) is a rodent zoonotic orthopoxvirus (OPXV) that has recently re-emerged globally with unprecedented human-to-human transmission. The 2022 global outbreak strain (Clade IIb, lineage B) derives from Clade IIb lineage A strains that have established human circulation but remain endemic to West Africa. Lineage B isolates from 2022 have a unique mutational footprint, likely from host APOBEC3 enzymes and hence indicative of sustained transmission amongst humans, but the biological consequences of which remain unknown. Here we show that despite only 46 nucleotide differences, the global 2022 strain has attenuating phenotypic changes relative to an ancestral lineage that has not spread globally. We found that global and endemic MPXV replicated equivalently in relevant cell types, but the global lineage B virus had defects in long-range spread. Global MPXV also displayed defects in innate immune control, including IFNβ induction, increased innate immune signalling and MAPK gene expression, and a reduced capacity to suppress interleukin-1β-mediated immune responses. Finally, the global strain had reduced virulence in a mouse model of MPXV infection relative to the ancestral endemic strain. Our work demonstrates biological differences between human circulating MPXV lineages that correlate with clinical observations documenting reduced in-host viral dissemination and disease severity for lineage B. Our data also reveals that the APOBEC3-like mutational footprint in globally circulating MPXV is not only a signature of sustained human transmission but also drives phenotypic changes.

Virus Symposium: Viruses and Cancer

Invited: Rationally designed combination therapies to enhance the efficacy of oncolytic reovirus.

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Abstract

Cancer treatment has improved over recent decades; however, therapeutic challenges remain for many cancer types. These include: (i) drug resistance/relapse and tumour immune evasion, which prevents chemotherapy and/or immunotherapy from working, and (ii) treatment-related adverse events, which prevent potentially effective therapies from being used in elderly or frail patients. Therefore, novel therapies are required to overcome these clinical challenges and improve patient outcomes.

Oncolytic viruses (OVs), such as reovirus, utilise multiple mechanisms to exert their anti-cancer effects, including direct oncolysis, bystander cytokine killing, activation of innate (Natural Killer [NK] cell) and adaptive (T cell) anti-tumour immunity, and modulation of the tumour microenvironment (TME). Importantly, reovirus has also been safe and well-tolerated in numerous clinical trials, making it an attractive addition to current clinical practice. Unfortunately, despite the promise of single agent reovirus treatment in numerous preclinical cancer models, results from early phase clinical trials have been disappointing. Therefore, our research has focused on identifying clinically relevant synergistic partners to increase reovirus efficacy across a range of cancer types. Published data demonstrating the direct and immunotherapeutic potential of reovirus will be presented alongside more recent (unpublished) findings showing that sub-toxic doses of: (i) apoptotic modulators (e.g., SMAC/BH3 mimetics) can augment reovirus bystander cytokine killing, and (ii) epigenetic modulators (e.g., histone deacetylase [HDAC] inhibitors) can enhance reovirus oncolysis and the induction of tumour- and reovirus- specific T cells. These pre-clinical findings support the development of phase 1b clinical trials investigating the therapeutic potential of these rationally designed combination therapies.

Invited: Identifying novel therapeutic targets in HPV-associated cancers

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Abstract

Human papillomaviruses (HPV) are a major cause of cancer worldwide, and are the primary cause of almost all cervical cancers and a growing number of cancers of the head and neck (HNCs). The viral proteins E6 and E7 are the main proteins responsible for HPV+ cancer development. Unfortunately, despite the development of vaccines for HPV infection, there are no HPV-specific treatments currently available, with the most common treatment for HPV+ cancers being chemotherapy or radiotherapy. Interestingly, HPV+ cancers respond much better to these treatments than similar HPV- cancers, but the side effects can be debilitating. The reasons why HPV+ cancers respond better to current treatments is unclear. Thus, identifying why HPV+ cancers response better to these treatments may allow us to find better treatments for HPV- cancer and reduce the side effects observed in HPV+ cancers.

Our work is focused on identifying novel therapeutic targets in HPV-associated cancers, with a particular focus on targets that sensitise these cancers to current chemotherapies and radiotherapy. In this talk, I will highlight our recent work that identified several mitotic kinases and ubiquitin pathway genes that are involved in promoting therapeutic resistance in HNC, highlighting their potential as novel sensitising agents in both HPV+ and HPV- HNC. I will also discuss how our ongoing studies are further probing the molecular differences between HPV+ and HPV- HNC, with the aim to identify novel HPV-specific therapeutic targets.

Invited: Development of “precision virotherapies” for the systemic treatment of cancer.

Alan Parker ORCID iD

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Abstract

Oncolytic viruses (OV) hold immense clinical potential for the treatment of cancer. As biologics, they hold the capacity to replicate selectively within the tumour microenvironment, amplifying the therapeutic at the point of need, inducing immunogenic cell death and overexpressing engineered therapeutic transgenes, such as potent immunostimulatory payloads, within the local tumour environment. To date, however, results from clinical trials have been underwhelming, largely due to the inability of OV to selectively transduce cancerous cells and leave healthy cells and tissues uninfected following delivery in vivo.

Our laboratory has taken a structural virology-based approach to rationally engineering adenoviruses better suited to intravenous tumour targeting applications. We have defined several interactions between host proteins and receptors that underpin natural adenovirus tropism and pathogenesis. Through selective modification of the capsid proteins, we have been able to generate basal “NULL” platform vectors suitable for retargeting applications. Using these “NULL” platforms, we have further engineered in peptides targeting tumour antigens, which provide an alternative means of cell entry and infection of these “precision virotherapies”.

Our first precision virotherapy, $Ad5_{NULL}$-A20 (also known as Trocept or ATTR-01) is selective for the tumour antigen and marker of tumour aggression, αvβ6 integrin. Additional modifications enable replication of this agent selectively within αvβ6 integrin positive cells, and ATTR-01 is additionally modified to overexpress a full-length anti-PD-L1 antibody within tumour cells following intravenous delivery. ATTR-01 has undergone IND enabling studies and is due to enter a first in human dose escalation clinical trial in αvβ6 integrin positive carcinomas in early 2025.

Role of the IE1 and pp65 Proteins in Cell Transformation and Oncomodulation by Human Cytomegalovirus

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Abstract

Although not currently classified as a carcinogen by the International Agency for Research on Cancer, human cytomegalovirus (HCMV) has been implicated in the pathogenesis of glioblastoma and several tumours of epithelial origin, including triple-negative breast cancer. The most commonly detected viral proteins in HCMV-associated cancers include immediate early protein 1 (IE1) and the tegument protein pp65.

To investigate a possible role of IE1 and pp65 in oncogenesis, we transduced mesenchymal stem cells, known to be susceptible to transformation by adenovirus oncogenes, with lentiviruses expressing HCMV IE1 and/or pp65. Unlike adenovirus E1A and E1B, IE1 and pp65 did not transform primary cells, either alone or in combination with the adenovirus oncogenes. Instead, both IE1 and pp65 showed inhibitory effects on cell transformation.

To assess whether HCMV affects cancer progression via 'oncomodulation', a low-malignant breast cancer cell line (MCF-7) was transduced with IE1 and/or pp65. The transduced cells were analysed for changes in the transcriptome using bulk RNA sequencing. Among the top differentially expressed genes in IE1-expressing cells, many were associated with increased malignancy and resistance to paclitaxel, a first-line chemotherapeutic agent for breast cancer. We observed significantly higher cell viability in IE1-transduced cells compared to control cells after the addition of paclitaxel. This suggests that HCMV IE1 may play a role in increasing chemoresistance in breast cancer cells.

Our results do not support the idea that IE1 or pp65 contribute to oncogenic transformation of primary cells, but suggest an oncomodulatory role of IE1 in established breast cancer cells.

Unveiling the LncRNA Transcriptomic Profiles between HPV-Positive and -Negative Head and Neck Tumours

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Abstract

Human papillomaviruses (HPV) are a family of DNA viruses that, through persistent infection with high-risk subtypes, HPV-16 and HPV-18, can lead to cancers across various anatomical sites. Over the last decade, HPV-16 has surpassed tobacco smoking as the primary cause of oropharyngeal cancers, a subset of head and neck squamous cell carcinomas (HNSCCs). HPV-positive and HPV-negative HNSCCs have distinct clinical outcomes, though the molecular mechanisms driving these differences remain unclear.

Long non-coding RNAs (lncRNAs), known for their regulatory roles, are likely manipulated to drive transformation in both HPV-positive and HPV-negative cancers. Exploring the long non-coding RNA molecular signatures within patients may provide a promising 'starting point', for unravelling the specific biological-pathways contributing to discrete patient prognoses. This will offer potential biomarkers for predicting tumour aggression and enable more targeted therapies.

We conducted whole transcriptome sequencing on four HPV16-positive oropharyngeal and four HPV-negative oral cavity tumours, comparing each with healthy proximal tissue. Differential expression analysis highlighted 14 lncRNAs dysregulated between HPV-positive and negative HNSCCs. Five lncRNAs were selected based on a set of criteria to ensure lncRNA novelty and likelihood of promoting specific phenotypes. One lncRNA, FAM151B-DT, emerged as significantly upregulated in HPV-positive oropharyngeal tumours, with expression patterns reflected in HPV-positive HNSCC cell lines. This upregulation was also shown to be tied to HPV16-oncoprotein expression. This work represents the first analysis of differentially expressed lncRNAs in HPV-positive versus HPV-negative HNSCC, based on internally sourced and sequenced patient data.

Sp140L is a Novel Restriction Factor of Herpesvirus Infection

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Abstract

Epstein-Barr virus (EBV) is an oncogenic gammaherpesvirus that can transform B cells into lymphoblastoid cell lines in vitro. To establish latent infection, EBV must evade (or use) the intrinsic cellular immune processes. PML nuclear bodies (PML-NBs) are key components of this intrinsic response, repressing transcription of herpesviruses that lack appropriate countermeasures. The speckled protein (SP) Sp100 is a core component of PML-NBs that contributes to virus repression. The SP family also includes Sp110, Sp140 and (only in primates) Sp140L. Viral countermeasures to Sp100 include EBV protein EBNA-LP that binds and inhibits Sp100 and Herpesvirus Saimiri (HVS) ORF3 which degrades Sp100.

We previously showed that EBNA-LP-knockout EBV (LPKO) is unable to transform naïve B cells and exhibits reduced transcription of its genes. In single cell RNA-seq data, LPKO-infected B cells exhibited reduced viral gene expression and increased expression of interferon stimulating genes associated with Speckled protein expression. CRISPR knockout of either Sp100 or Sp140L (but not other PML-NB components) rescued outgrowth of LPKO-infected naïve B cells, and similarly, rescued transgene expression from ORF3-knockout HVS in human fibroblasts. By directing CRISPR mutations to specific exons, we found that truncations in either the SAND or PHD/bromo domain of Sp140L, or the SAND but not PHD/bromo domain of Sp100 – also rescued these phenotypes. Therefore, in human cells, Sp140L is an essential cofactor for the restriction of herpesviruses by Sp100. We hypothesise they work together to sense and repress transcription of nuclear viral DNA, and prevent transformation of naïve B cells.

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