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POSTER ABSTRACT BOOK

#Microbio26

MICROBIOLOGY SOCIETY

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Block A

Session : Actinomycetes: Industrial uses; cryptic pathways and novel antibiotic discovery

A001

From Cow to Cure: Exploring *Streptomyces* Biosynthetic Potential in the Bovine Gut

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Abstract

Shiga toxin–producing *Escherichia coli* (STEC) are significant enteric pathogens responsible for outbreaks of haemorrhagic diarrhoea. Conventional antibiotic therapy is contraindicated in STEC infections, as antimicrobial exposure can exacerbate toxin production, highlighting the urgent need for alternative therapeutic strategies.

Actinobacteria represent a prolific source of bioactive natural products, many of which exhibit anti-virulence activity against STEC. Recent studies indicate that Actinobacteria constitute a higher proportion of the faecal microbiota in cattle free from STEC compared with STEC-positive animals, suggesting that the bovine gastrointestinal tract may act as a reservoir of metabolites capable of attenuating STEC virulence.

To investigate this hypothesis, fifty bovine faecal samples were collected across the United Kingdom to characterise the *Streptomyces* population and evaluate its biosynthetic potential. An integrated approach combining metagenomic sequencing, culture-dependent isolation, and whole-genome analysis identified *Streptomyces* as a diverse and prominent component of the bovine microbiome. Forty-eight *Streptomyces*isolates were obtained and systematically assessed for biosynthetic gene clusters, enzymatic activity, and inhibitory or anti-virulence effects against STEC and ESKAPE pathogens.

These findings demonstrate that the bovine gut constitutes a rich reservoir of natural product–producing *Streptomyces*, providing a valuable resource for the discovery of novel bioactive metabolites with potential clinical applications and advancing understanding of their ecological role in modulating STEC virulence.

A002

Spectrophotometry characterisation of Antimicrobial properties of Actinomycetes from Marine habitats

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Abstract

Actinomycetes species from underexplored habitats are emerging as a promising source of novel bioactive compounds with diverse biological activities. This study aimed to isolate, characterise, and screen actinomycetes from the Ogun Waterside marine habitat for their antimicrobial potential. Soil and water samples were collected, with the soil samples pretreated and cultured on International Streptomyces Project (ISP2) and Minimal Salt Agar (MSA) media, supplemented with commercial antifungal agents and antibiotics to inhibit the growth of fungi and bacteria. The isolates were evaluated for their morphological, colonial, and biochemical characteristics. Clinical isolates of multidrug-resistant Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Klebsiella oxytoca were tested against the actinomycetes isolates. Cell-free extracts from actinomycetes that showed significant inhibition zones were analysed using Gas Chromatography-Mass Spectrometry (GC-MS). Fourteen actinomycete strains were isolated in total—thirteen from soil and one from water—identified as Streptomyces sp., Nocardia sp., and Micromonospora sp. All clinical isolates exhibited varying degrees of sensitivity to the actinomycetes species: Pseudomonas aeruginosa (12.5 mm), Klebsiella pneumoniae (14.5 mm), Klebsiella oxytoca (13 mm), and multidrug-resistant E. coli (10 mm). The GC-MS analysis revealed ten compounds, with notable metabolites including 12% acetic acid, 72% 2,3-butanediol, 2.47% 2,2-dimethyl-N-phenethylpropionamide, and 1.29% pyrrolo(1,2-a)pyrazine-1,4-dione. This study underscores the untapped potential of actinomycetes from underexplored marine habitats, particularly in the context of drug discovery. The findings contribute to the growing recognition of marine environments as a rich source of novel bioactive compounds with applications in medicine and industry.

A003

Streptomyces synthetic community Interactions reshape soil microbial communities.

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Abstract

In soil, nutrient limitation and fluctuating abiotic conditions drive intense microbial competition, with specialised metabolites playing a key role in shaping community dynamics.

Streptomyces is a genus of Gram-positive bacteria ubiquitous in soil. These bacteria are renowned for their ability to produce antibiotics and other relevant clinical drugs. In response to the environmental pressures in soil, Streptomyces synthesise bioactive specialised metabolites, which are used to support their survival and ecological success.

Other common soil bacteria include members of the phyla Bacteroidota, Bacillota and Pseudomonadota. Our research focuses on studying how interactions between different *Streptomyces* species and these soil-dwelling bacteria, specifically *Bacillus cereus*, *Pseudomonas koreensis*, and *Flavobacterium johnsoniae*, influence microbial community dynamics.

We aim to understand how S. venezuelae and *S. rimosus*, in the presence or absence of B. cereus, *P. koreensis* and F. johnsoniae, affect the composition and function of natural soil microbial communities. Ultimately, our goal is to uncover mechanisms that activate silent natural product biosynthetic pathways in *Streptomyces* species, and to provide insights for improving agricultural systems. To assess shifts in community composition and ecological function, we performed shotgun metagenomic analyses on compost microcosms inoculated with different combinations of these bacterial species.

A004

Extremophile Actinobacteria from the Atacama Desert: A Rich Source of Cryptic Antibiotic Biosynthesis

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Abstract

Antimicrobial resistance (AMR) poses a critical global health threat, necessitating the discovery of novel antibiotics from underexplored environments. The Atacama Desert, one of Earth's driest regions, harbours extremophile actinobacteria with unique biosynthetic potential. This study employed an integrated bioactivity-guided approach combined with whole genome sequencing to discover novel antibiotic-producing strains from Atacama soil samples.

We successfully revived 102 of 142 bacterial isolates collected from six sites at altitudes ranging from 3,018 to 5,046 metres. Direct bioreporter screening against *Escherichia coli*, *Bacillus subtilis*, and yeast identified 61 isolates exhibiting antibacterial activity. These bioactive strains were prioritised for whole genome sequencing, yielding 49 high-quality genomes predominantly comprising *Streptomyces* species (n=47), with around 21 strains representing putatively novel species based on phylogenetic analysis.

Biosynthetic gene cluster (BGC) mining using antiSMASH v7.1.0 identified 1,434 BGCs across sequenced genomes, including non-ribosomal peptide synthetases (63), polyketide synthases (54), and ribosomally synthesised post-translationally modified peptides (284). BiG-SCAPE network analysis revealed that 60% of the 501 gene cluster families were singletons, indicating substantial strain-specific biosynthetic diversity unexplored in existing databases. Notably, isolate H2 harbours a unique SAM_SPASM radical SAM domain-containing RiPP cluster with strong potential for producing novel sulfur-crosslinked natural products.

Genome-informed secondary screening and LC-MS/MS metabolomic profiling of prioritised strains confirmed bioactivity and revealed multiple novel mass signatures in bioactive extracts, validating the presence of uncharacterised antimicrobial metabolites. This integrated approach demonstrates that combining phenotypic screening with genome mining efficiently identifies cryptic biosynthetic pathways in extremophile actinobacteria for antibiotic discovery.

A005

Genome–Metabolome Integration Reveals Antimicrobial Biosynthetic Potential in a Novel Streptomyces from the Scottish Machair

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Abstract

A soil sample collected from the rhizosphere of Machair vegetation at Achmelvich Bay, Scotland, harboured a diverse microbial community dominated by Proteobacteria, followed by Actinobacteria, Acidobacteria and Bacteroidetes. From this habitat, a *Streptomyces* isolate displaying antimicrobial activity was obtained. Genome sequencing revealed an 8.34 Mb genome (G + C 71.5%) assembled into four contigs (N50 = 8.1 Mb). Digital DNA–DNA hybridisation (67%) and a G + C difference of 0.2% compared with *Streptomyces durocortorensis* suggest that the strain represents a novel *Streptomyces* species. antiSMASH 8 analysis identified 34 biosynthetic gene clusters, including several NRPS and hybrid PKS–NRPS systems showing low similarity to characterised clusters.

Metabolomic profiling (LC–MS/MS) detected multiple metabolites consistent with NRPS-type biosynthesis and putatively corresponding to predicted genomic regions. Crude extracts exhibited antibacterial activity against the Gram-positive bacterium *Bacillus subtilis*. Together, these findings integrate genomic, metabolomic and bioactivity evidence to support the isolate as a promising candidate for antimicrobial secondary metabolite discovery and highlight coastal Machair soils as underexplored environments for antibiotic discovery.

A006

Unearthing environmental streptomycetes with antibacterial activity against ESKAPE pathogens: an undergraduate student-led investigation

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Abstract

Antibiotic resistance amongst ESKAPE pathogens, which are responsible for many hospital-acquired, multidrug-resistant infections, remains a significant global health challenge. As treatment options diminish, the discovery of new antimicrobial compounds is becoming more critical. Actinobacteria, particularly *Streptomyces* spp., have a long track record as antibiotic producers, yet their continued recovery, often from unconventional environmental niches, suggest their potential is far from exhausted. Through the *Antibiotics Unearthed* initiative, undergraduate Honours students isolated putative *Streptomyces* spp. from soils, lichens, and outdoor murals. Isolates displaying typical streptomycete traits were screened for antibacterial activity against ESKAPE pathogens (*Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) using cross streak, spot-overlay, and agar plug assays. Active isolates were confirmed as *Streptomyces* spp. through 16S rRNA gene sequencing and screened for key biosynthetic gene clusters (PKS I, PKS II, NRPS) by endpoint PCR. Strains demonstrating inhibition were further investigated in axenic and co-culture conditions to assess the persistence and induction of antibacterial activity. Cell-free supernatants from liquid cultures were tested over the duration of incubation, revealing variable activity profiles. This work underlines the continued promise of environmental *Streptomyces* as antibiotic producers whilst showcasing the benefits of authentic, semi-structured research projects for enhanced student experience and scientific output – a theme further explored in our companion teaching-reflection poster.

A007

Regulation of Chloramphenicol Biosynthesis in *Streptomyces venezuelae*

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Abstract

Streptomyces venezuelae is the native producer of chloramphenicol (Ehrlic et al., 1948) and is a genetic model for the study of *Streptomyces* bacteria. The study of the regulation and biosynthesis of chloramphenicol represents an opportunity to obtain a complete understanding of how *Streptomyces* bacteria control both the temporal and spatial dynamics of natural product synthesis.

The regulation of chloramphenicol biosynthesis is controlled by numerous transcriptional regulators. The cluster situated regulator CmlR (Fernández-Martínez et al., 2014) positively activates transcription of the biosynthetic genes and was previously shown to be able to switch on chloramphenicol production when expressed constitutively. While there are also the pleiotropic regulators MtrA (Som et al., 2017) and AdpA (Wolanski et al., 2025) that can globally effect gene expression and also both specifically bind to promoters in the chloramphenicol cluster. MtrA binds within the chloramphenicol cluster between the genes *cmlN/F* that encode transporter proteins. Deletion of *mtrA* switches on constitutive chloramphenicol production, causing a huge increase in production of the biosynthetic enzymes and the antibiotic.

Here we have generated *cmlR* and *adpA* mutants in both the wildtype background of *Streptomyces venezuelae* and also in the *mtrA* mutant. This has allowed us to test the dependency on both CmlR and AdpA for the output of chloramphenicol production in the *mtrA* mutant. This gives us insight into the overall multi-level regulation that can occur in a genetic host for production of a desirable natural product and offers an insight into how *Streptomyces* can be manipulated for industrial uses.

A008

Metagenomic Profiling and Isolation of Novel Actinobacteria from Puck's Glen, Scotland, Reveal Potential Antibiotic Producers

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Abstract

Actinobacteria remain a prolific source of novel antibiotics, yet temperate forest soils are comparatively underexplored. In this study, Oxford Nanopore Technologies (ONT) long-read metagenomic sequencing was integrated with culture-based isolation to characterise actinobacterial diversity and biosynthetic potential in Puck's Glen, a humid coniferous forest in western Scotland.

Metagenomic profiling identified over 300 bacterial genera, with Actinobacteria representing the most abundant and diverse phylum. Dominant taxa included *Streptomyces*, *Micromonospora*, *Trebonia*, and other Actinobacteriota, many recognised for secondary metabolite production. Taxonomic classification and Krona visualisation guided selective cultivation, enabling isolation of multiple actinobacterial strains.

Preliminary antimicrobial screening using agar diffusion assays demonstrated inhibitory activity of several isolates against Gram-positive test organisms, indicating promising biosynthetic potential. This integrative approach—combining culture-independent metagenomics with culture-dependent screening—provides a robust framework for linking microbial community composition to functional bioactivity.

The findings establish Puck's Glen as a previously uncharacterised reservoir of antibiotic-producing Actinobacteria and demonstrate the utility of ONT-based metagenomics in guiding bioprospecting efforts. The results highlight the potential of temperate soil microbiomes for natural product discovery and their relevance to addressing the global antimicrobial resistance crisis.

Session : Education and Outreach Symposium

A009

Enhancing Communication Skills in Microbiology Through Outreach

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Abstract

Effective science communication is essential for modern microbiologists, especially as public understanding increasingly shapes global health outcomes. Yet, traditional curricula often emphasize technical skills over communication, leaving students lacking lay communication competency. In our Medical Microbiology MSc at the University of Manchester, we introduced an assessment where students designed an outreach activity and lay poster, to deliver to the general public, communicating key microbiological concepts.

Students were given a questionnaire pre and post completion of the assessment to evaluate the benefit of this type of assessment to communication skills. The questions evaluated students understanding of lay language and their confidence in communicating science to the public, using either yes/no questions or ratings on a scale from 1-5. Roughly 70% and 50% of students completed the pre- and post-assessment evaluation respectively.

65% of our students had not previously participated in an outreach activity. Number of students with high confidence in their communication skills didn't differ greatly pre- and post-assessment (rating >3), but the number of student rating confidence at 1 or 2 did reduce post-assessment. All students were aware what lay language meant post-assessment (100%), compared to 58% pre-assessment. An increase in the number of students reporting high confidence in communicating using lay language was also observed (84% pre vs 100% post, rating >3).

This assessment highlights the value of integrating science communication into microbiology education, particularly in improving students' confidence and ability to use lay language.

A010

Antimicrobial Resistance – The Graphic Novel: establishing an interdisciplinary project team

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Abstract

An interdisciplinary collaboration was established between the Centre for Education and Students (Shaw), the National Centre for Food Manufacturing (Crewe) and the Lincoln School of Design (Leary) at the University of Lincoln. The aim, aligning to Global One Health, was to enhance communication and dissemination of complex topics by creating a graphic novel about antimicrobial resistance (AMR).

Science fundamentals, suggested dialogue and AI-generated images were developed to communicate the envisaged content to the illustrator for the first chapter; the impact of AMR on future global health. Early meetings revealed a layer of 'hidden scientific knowledge'; a disciplinary gap that needed to be overcome for the illustration to accurately capture the scientific concepts. Additional background context was written, and time allocated during meetings to discuss scientific concepts. These strategies helped establish clearer cross-disciplinary communication and supported a more cohesive creative process.

The team have co-created two further chapters; the use of antibiotics in agriculture, and an exploration of phage therapy. Optional additional resources have been created to support educators in incorporating the graphic novel into their teaching for students aged 12 and upwards. These resources are available with an Open Access Creative Commons licence, and a limited run of print copies are available thanks to funding from the Microbiology Society.

The project has successfully fostered a collaborative team culture grounded in an ethic of reciprocity, enabling co-construction of novel educational resources. Surveys to evaluate the experiences of educators and students are being shared, with findings to be reported in future publications.

A011

Science: Live on Stage – bringing science to schoolchildren through theatre

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Abstract

In April 2023, collaboration began between Lincoln Arts Centre (Anderson), Lincoln School of Creative Arts (Sanchez and Smith) and the National Centre for Food Manufacturing (Crewe), all based within the University of Lincoln. The aim was to bring extraordinary science to life in a 50-minute performative, educational lecture for schoolchildren and families called Science: Live on Stage. Over a period of seven months, a script was written, a theatrical vision was devised, and plans for set/props (Chambers), lighting (Marcinek and Bradbury), animation (Peverill) and sound (Cable) were developed and rehearsed. This activity culminated in a publicly facing run of shows in November/December 2023.

The script focused on how scientific understanding of microbiology has advanced through time starting with the Big Bang through the development of microorganisms, the discovery of Escherichia coli, the role that E. coli plays in human health and disease, and its importance as a laboratory model now and into the future. Throughout the show, the narrator-lecturer (Crewe) was 'helped' by a somewhat clumsy 'lab assistant' (Sanchez) who engaged the audience via interactive means: bringing volunteers onto stage for demonstrations, sharing sweets and 'making mistakes' which the narrator had to correct.

Audience testimonies included "I never thought I'd belly laugh at microbiology" and "I wish my students would be this enthusiastic in school, I've never seen them so excited". The project has now developed into an annual series produced by LAC: the 2024 show was about the human brain, the 2025 show is about sport science.

A012

Microbiology for Apprentices: linking KSBs to real-world experiences

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Abstract

Apprenticeships are programmes of study for learners in full-time employment. Within an apprenticeship programme, knowledge (K) is delivered by
a training provider and development of skills (S) and behaviours (B) are supported by both the training provider and the employer. Learners develop their KSBs in a structured way across their practical period, which is mapped within a training plan. At the end of this practical period, when the learner is deemed competent against the standard, the learner enters Gateway, after which point they draw everything together to engage with their End Point Assessment (EPA). Successful completion of their EPA results in apprenticeship certification and, in the case of degree apprenticeships, simultaneous award of a credited University qualification.

For several apprenticeship standards relating to the food industry, microbiology is a critical component, where knowledge such as “the microbiology and microbial ecology in a range of foods” and “microbiological testing procedures relevant to food safety and quality” must be acquired, and skills such as “interpret microbiological results” and “review and select relevant scientific techniques taking into account cost and practicalities” need to be developed by the learner.

This (talk/poster) will aim to demystify some of the terminology around apprenticeships, explain how a university can fulfil the role of training provider and provide some examples of how KSBs linked to microbiology are developed through teaching, learning and assessment strategies delivered online and in person and within both a university environment and a business location.

A013

The Development of a Microbiome Based Springer Project to Develop Readiness for Capstone Projects

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Abstract

The Capstone Research Project is the pinnacle of an honour's degree in Biomedical Sciences. While some students may undertake short research placements, or a placement year between their second and third year of study, most students do not get an opportunity to pursue a structured research project prior to their Capstone project. To bridge this gap, within the University of Bradford biomedical sciences course, springer projects have been introduced as part of the laboratory and professional skills modules in years 1 and 2. These springer projects focus on the development and application of research skills. The second-year project follows a skin microbiome theme with students designing and investigating research questions around the abundance, composition or resistance within the skin microbiome. A guided approach to the projects is taken starting with a context and biological introduction, multiple personal academic tutor facilitation sessions to assist with project design, health and safety, and analysis. The intention is to enhance students' confidence and understanding of research ahead of their high-stakes Capstone project. In addition, this reinforces and builds upon learning covered in the introductory and medical microbiology modules. The first cohort will be undertaking Capstone projects in spring 2026. This presentation will discuss the scientific development and feasibility of the project.

A014

Developing innovative educational resources for European COST Actions

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Abstract

A COST Action is a bottom-up, interdisciplinary, pan-European research network funded by the European Cooperation in Science and Technology (COST) program, designed to foster collaboration and knowledge exchange among researchers and innovators across various science and technology fields. COST Actions of interest to microbiologists include CA22109 'Medicinal plants for animal health care: Translating tradition into modern veterinary medicine (MedPlants4Vet)', CA23152 'Building Consensus on Biofilm Regulatory Decision Making (RegulatoryToolBox)' and CA20130 'European MIC Network – New paths for science, sustainability and standards (Euro-MIC)'. The just-finished Euro-MIC has established an impressive range of educational e-resources 'MIC Courses' that were highly appreciated by participants across industry and academia which would still be of use to microbiology educators and learners alike. This presentation will introduce the two active COST Actions MedPlants4Vet and RegulatoryToolBox, and will explain how interested microbiologist can join (for free) and contribute to the networks. We will focus here on the development, delivery and evaluation of educational tools and events. For MedPlants4Vet, a workshop named 'Herb power for animal health: from tradition to legislation' was run and attended by mostly veterinary students or practitioners from many different European countries including Turkey, Greece, Croatia, Romania and Italy. Games and other innovative teaching approaches were efficiently employed. 95% of the attendees found our lectures useful and enjoyable. More such workshops are planned for the near future. RegulatoryToolBox has only been active for one year, in which two general meetings and one educational event around existing regulatory frameworks for biofilm-targeting products have been delivered.

A015

Culturing for the win: a student course to assess underrepresented taxa in isolate collections

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Abstract

Culturing projects, in our example mosquito microbiomes, are still mainly based on only basic microbiological culturing conditions, LB medium incubated at 37C overnight. This is favouring growth of already well-described taxa, especially Enterobacteriaceae, whilst a large part of the microbiome is not able to grow under these conditions or rapidly outcompeted by fast-growing taxa well-adapted to these conditions. The mosquito microbiome is of high interest given its impact on the transmission of important pathogens (including arboviruses such as dengue or chikungunya virus, and *Plasmodium* spp.). Based on mosquito samples derived from different times of the mosquito life stage known to undergo significant changes in microbiome composition we designed a culturing approach targeting the main taxonomic groups known to occur based on a historied body of work describing 16S rRNA sequence diversity, and recent insights on eukaryotic taxa via 18S rRNA and ITS sequences. We used eight agar types (six selective for taxa of interest, two broad selection) under a total of ten conditions, trying to mirror the mosquito environment (25C - 30C, 1-7 days of growth). This showed a large diversity of bacterial and potential fungal growth, which we monitored via colony counts and growing single colonies to perform basic phenotypic tests and 16S rRNA sequencing and analysis, and select isolates will undergo whole-genome sequencing and analysis. This will provide insights into understudied taxa in the microbiome as well as in broader sense provide insights and genome data for understudied microbial taxa, of interest for the microbial community.

A016

From Data to Doodles: Communicating Science Through Sketchnotes

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Abstract

An increase in digital and graphic design has allowed science communication to evolve beyond traditional formats, with visual storytelling emerging as a powerful and inclusive tool to bridge the gap between researchers and the public. This project explores the use of sketchnotes- a creative fusion of art and science that transforms complex research into accessible, engaging visual narratives. In this approach, researchers collaborate with an artist to identify the key concepts and messages of their work, which are then illustrated through hand-drawn visual notes combining text, diagrams, and images. The process itself acts as a reflective exercise, encouraging researchers to clarify their ideas, prioritise key findings, and develop stronger communication skills.

Sketchnotes distil intricate scientific concepts into visually appealing formats that attract attention, simplify information, and make research more approachable to broader audiences. By visually representingprocesses that are often difficult to convey through words alone, this method enhances comprehension, encourages dialogue, and inspires curiosity among both scientific and non-specialist viewers.

The poster will showcase examples of collaborative science to art pieces and include testimonials from researchers reflecting on how the process helped refine their science communication and self-awareness of their work. Beyond outreach, this practice promotes inclusivity and accessibility in science, bringing research into everyday spaces—from classrooms to homes—thereby destigmatising and humanising science. By integrating creative visualisation into the research culture, science sketchnotes offer a versatile and impactful way to share discoveries, foster collaboration, and engage the public imagination.

A017

Bug-buster: an outreach activity to raise awareness about antimicrobial resistance.

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Abstract

Antimicrobial resistance (AMR) is a growing global health threat, driven in part by misuse and overuse of antibiotics in different settings (human, animal and environmental). This outreach activity aims to increase public awareness of AMR, promote responsible antibiotic use, and encourage community engagement in prevention efforts.

For this activity, we manufactured bacteria (rod-shape) with green, orange and red foam. On the green bacteria (sensitive), we added both adhesive magnets and protruding pieces of jute thread. On the orange ones (resistant to one antibiotic), we added only magnets or jute thread while we didn't add anything to the red ones (multidrug resistant). For the "antibiotics", we used a rod with a magnet or a hook attached using some thread to "kill" (fish) our bacteria.

We asked the participants to start "killing" the bacteria and to sort them by colour during a limited time (1-2 minutes). They ended up "killing" all the green and orange bacteria but none of the red ones. This served us as an introduction for the discussion (how bacteria become resistant to antibiotics, causes of the AMR and what can they do as the community to prevent AMR). This activity has been successfully presented for 4 years to the Science Fair of the University of the Balearic Islands (Spain).

Through this interactive activity and the subsequent discussion, participants gained a clearer understanding of how AMR arise and its consequences as well as everyday actions that can help slow the spread of resistant infections.

A018

From Report to Action: Interpreting Antimicrobial Susceptibility in Everyday Clinical Practice

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Abstract

Background:
Clinician interpretation of antimicrobial susceptibility reports is essential for appropriate infection management. In 2019, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) redefined the "I" category from *intermediate* to *susceptible, increased exposure*, indicating that therapeutic success is likely with optimised dosing. At OLOLD, this category is reported as "S*" to address potential prescriber reluctance to use agents labelled as "I."

Aim/Methods:
A questionnaire was distributed to clinicians across multiple specialties and training levels to evaluate understanding of antimicrobial susceptibility categories.

Results:
Forty-four responses were received from seven specialties, including medical (38.6%), surgical (18.2%), and orthopaedic (11.4%) disciplines. Respondents were from all training levels, with 18.2% of respondents identifying as consultants. Most respondents (95.5%) would not prescribe an antibiotic reported as resistant. Only 79.5% would prescribe an antibiotic reported as susceptible on a urine culture. Willingness to prescribe was higher for agents categorised as "S*" (59.1%) compared with "I" (43.2%), despite identical definitions.

Conclusion:
Significant confusion persists regarding the "S*" and "I" categories, with inconsistent interpretation across all specialties and training levels. The finding that nearly one in five clinicians would not prescribe an antibiotic reported as susceptible highlights the need for enhanced education and continued support from Clinical Microbiologists. We plan further investigation through educational initiatives, followed by a repeat survey. The study will also be extended to other hospitals and include evaluation of alternative reporting mechanisms to identify strategies that improve clinician understanding and optimise antimicrobial use.

A019

Reflecting on Honours Project Delivery through *Antibiotics Unearthed*: A Semi-Structured, Group Supervision Approach for Equitable, Research-Driven Learning and Curriculum Sustainability

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Abstract

We reflect on the evolution of a semi-structured, group-based model for Honours project delivery, initially piloted through the Antibiotics Unearthed initiative, funded by the Microbiology Society. Traditional one-student, one-supervisor projects are susceptible to inequities in student experience and isolated learning. To address these challenges and growing resource pressures, a thematic, group-based approach was developed, enabling students to pursue individual research strands within a shared framework supported by multiple supervisors. The approach fosters authentic, peer-supported research experiences, incorporating structured lab blocks, shared protocols, skills and data analysis workshops, and collaborative data discussions, alongside independent working to balance autonomy with guidance. Reflections from students, academics, and technical staff highlight multiple benefits. Students report increased clarity, confidence, resilience, and sense of community. Supervisors note consistent oversight, workload efficiencies, and opportunities for sharing good practice, while technicians observe more efficient resource use and safer lab environments. Challenges include ensuring student engagement within a structured, time-constrained schedule and balancing student instruction with independence and initiative. Overall, this model delivers authentic research experiences and equitable learning whilst managing resources more effectively. Positive outcomes have led to adoption across all lab-based disciplines in the undergraduate biological sciences provision, demonstrating scalability and sustainability. This practice-led intervention also demonstrates how a funded initiative can act as a springboard for curriculum enhancement. It may be of particular interest to microbiology educators seeking to foster peer learning, group teaching or strengthen curriculum sustainability.

A020

Immersive virtual reality in second-level and higher education: a partnered narrative on the challenges and opportunities for STEM engagement

Jerry Reen, Niall O'Leary

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Abstract

Digital education in the life sciences has seen several remarkable advances in recent years, not least with the advent of visual and immersive technologies that bring into focus the conceptually challenging abstract concepts that underpin molecular biology and the life sciences. In some cases, limitations in visualizing and modelling these concepts can prove to be a barrier to learning. Providing new entry points to learning through 'doing' or 'seeing' could prove to be a significant enhancer of engagement, unlocking hidden potential in our student cohorts and increasing the uptake of science as a subject of choice in higher education. In this study, the third in a series of pedagogically grounded research explorations of immersive virtual reality in STEM, second-level education teachers and higher education practitioners worked in partnership to explore the current state of the art around design and integration of immersive virtual reality simulations for the teaching of microbial and human cell structures in the classroom. We also considered the wider application of virtual reality and immersive learning technologies for science, technology, engineering and mathematics engagement and learning. Building on prior discoveries from student partnered co-creation of immersive reality simulations, the ultimate goal is to signpost the integration of visual immersive learning in the STEM curriculum.

Session : Eukaryotic Microbes in Health and the Environment

A021

Biofilm-Resistant Dental Implants: Exploring Omega-3 Fatty Acids as a Functional Coating

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Abstract

Biofilms are central to development of oral diseases, particularly dental caries, which exhibits heightened resistance to conventional treatments. This study investigated antibiofilm properties of Omega-3 (n-3) fatty acids Eicosapentaenoic acid (EPA, C20:5) and Docosahexaenoic acid (DHA, C22:6) against *Streptococcus mutans*ATCC 25175, a primary cariogenic pathogen.

Using a modified McBain medium simulating salivary conditions, bacterial growth was assessed at concentrations of 250, 100, 50, 25 and 10 µg/ml. Minimum biofilm inhibitory concentration (MBIC) was determined on titanium discs preconditioned with artificial saliva and assessed using colony enumeration, crystal violet staining and scanning electron microscopy (SEM). Cytotoxicity and proliferation effects on TR146 oral cells were investigated using lactate dehydrogenase (LDH) release and XTT assays. Additionally, n-3 incorporated medical-grade shellac coatings were developed and evaluated for biofilm prevention.

EPA and DHA significantly inhibited *S. mutans* growth and biofilm formation at 250-100 µg/ml. EPA was most effective in the presence of artificial saliva, with 25 µg/ml effecting growth and biofilm formation when exposed to salivary components. DHA was less effective at the same concentration, suggesting that salivary components may buffer or diminish its antibiofilm activity. Lowest concentrations (10 µg/ml) displayed no significant antibiofilm activity however, exhibited limited cytotoxicity compared to chlorhexidine. Shellac coatings containing Omega-3's effectively reduced surface biofilms in a dose-dependent manner, with SEM confirming biofilm inhibition at highest concentrations.

Omega-3 fatty acids, particularly EPA, exhibited antibiofilm activity against *S. mutans*, offering a natural, low-cytotoxicity strategy for dental implant coatings preventing secondary caries and dental implant failure.

A022

Performance and Cost-Utility of Non-Culture Diagnostics for Invasive Aspergillosis in a Resource-Limited Setting: A Prospective, One-Year Study in Peshawar, Pakistan

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Abstract

Background: Invasive Aspergillosis (IA) remains a leading cause of morbidity and mortality in immunocompromised patients, particularly in Low- and Middle-Income Countries (LMICs) like Pakistan, where delayed diagnosis due to limited culture facilities and high costs of treatment drive poor outcomes. We assessed the diagnostic accuracy and comparative cost-utility of multiple non-culture assays in Peshawar to identify the most sustainable rapid diagnostic strategy.

Methods: This prospective, one-year (Jan–Dec 2025), observational study at a large tertiary hospital in Peshawar, Pakistan, enrolled 485 consecutive high-risk patients suspected of IA. All subjects were tested using serum Galactomannan, plasma Beta-D-glucan, Aspergillus Lateral Flow Assay (LFA), and IgG/IgM serology. Definitive diagnosis was composite EORTC/MSGERC criteria. Accuracy metrics (Sensitivity, Specificity) and cost-per-result were compared.

Results: Of the 485 patients, 62 (12.8%) met the criteria for IA. GM showed the highest sensitivity (79.0%) at a cost of 35$. The LFA was the most cost-effective at 12$, achieving the highest specificity (97.7%) but lower sensitivity (61.3%). BDG (45$) and serology (20$) performed inferiorly with sensitivities of 74.2% and 50.0%, respectively. Notably, the combined use of LFA and GM yielded a high overall sensitivity of 90.3%.

Conclusion: In the high-burden, resource-constrained setting of Peshawar, the Aspergillus LFA represents an economically viable first-line approach for rapid IA detection due to its high specificity and low cost. While Galactomannan remains the most sensitive individual assay, its higher expense challenges sustainability. These findings support integrating LFA-based assays into LMIC diagnostic algorithms to accelerate antifungal stewardship.

A023

Testing reference-free tools for phylogenetic clustering in malaria parasites

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Abstract

Malaria is one of the most common infectious diseases worldwide, with 263 million cases and 507,000 deaths in 2023. Multiple species of *Plasmodium* cause malaria in humans, with *P. falciparum* being the most common and deadliest. Inferring genetic clusters is important for epidemiology, for identifying the specific species causing disease, and for tracking transmission.

Methods currently rely on differences in particular genetic markers, such as SNPs in antigen genes and microsatellites. There are also comparisons at the whole genome level, including studies in specific countries and the Malaria Genomic Epidemiology Network, which has compared thousands of genomes from *P. falciparum* and *P. vivax*. However, these genomics methods are commonly based on comparisons to a single reference genome. Such methods require computationally intensive bioinformatics pipelines and substantial expert knowledge, making them potentially unsuitable for resource-poor settings with the highest disease burden.

To combat this, we tested whether a reference-free method, SKA2, could be used to cluster *Plasmodium* genomes at different levels of diversity. To do this, we collected a set of 89 genomes from 16 *Plasmodium* species infecting humans and animals, assembled at the chromosome or complete level. DNAdiff was used to establish a ground truth SNP distance between samples and then compared to SKA2 using both simulated reads and re-assembled genomes as inputs. Computational resources for each method were also assessed to determine if this pipeline is feasible in a resource limited setting.

A024

Elucidating the unknown diversity and functional roles of eukaryotic microorganisms in subseafloor ecosystems.

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Abstract

The deep sea is the largest biosphere on Earth and a key frontier in ecological research. The subseafloor, composed of diverse and largely unexplored environments, harbors abundant microbial life. Despite limited access, it is estimated that around 80% of Earth's microbial biomass resides in the deep subsurface, making it one of the planet's largest microbial reservoirs. These environments are particularly important such as marine sediments acting as major carbon sinks and associated microbial activity playing a crucial role in global biogeochemical cycles. However, the diversity and ecology of microorganisms, particularly microbial eukaryotes, remain critically understudied due to the scarcity of cultured representatives and genomic data. Current knowledge relies heavily on metabarcoding, which lacks resolution due to limited reference databases and the high proportion of unknown and undescribed taxa. This project addresses these critical knowledge gaps through an integrative approach combining microbial cultivation and whole-genome sequencing to investigate subseafloor microbial eukaryotes. Our research addresses three principal scientific objectives: (1) characterizing the taxonomic diversity and phylogenetic relationships of subseafloor eukaryotes to reveal hidden biodiversity and evolutionary patterns; (2) elucidating their functional capabilities and metabolic strategies through genomic analyses to understand their roles in biogeochemical processes; and (3) investigating their potential ecology within these extreme environments. This research aims to advance our current understanding of subseafloor ecosystems and their roles in global biogeochemical cycles, with implications for biology, ecology, evolution, and biotechnology, ultimately illuminating one of Earth's most enigmatic and significant microbial frontiers.

A025

Airborne Fungal Diversity and Azole-Resistant Aspergillus fumigatus in a Care Home Environment

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Abstract

Airborne eukaryotic microbes form an important yet understudied component of indoor microbiomes, particularly in healthcare and long-term care environments. This study explored the diversity and seasonal dynamics of airborne fungi within a care home setting, with a focus on the opportunistic pathogen *Aspergillus fumigatus*. Air samples collected across four seasons were analysed using internal transcribed spacer (ITS) amplicon sequencing, revealing diverse and dynamic fungal communities. Distinct temporal and spatial variations were observed, with recurrent detection of genera such as *Aspergillus, Alternaria*, and *Cladosporium*. Diversity metrics, including Shannon and Chao1 indices, highlighted significant shifts in community composition between locations and seasons. Complementary culture-based analyses combined with MALDI-ToF identification and azole susceptibility testing confirmed the presence of *A. fumigatus* isolates exhibiting triazole resistance. Whole-genome sequencing of resistant isolates identified mutations in genes associated with azole resistance, providing insight into local environmental reservoirs of clinically relevant strains. Ongoing metagenomic analyses further extend this work by linking fungal diversity with antimicrobial resistance determinants. Collectively, these findings underscore the importance of integrated molecular and culture-based surveillance to monitor airborne fungal pathogens and resistance emergence in healthcare environments.

A026

Two decades of Trichophyton species isolates in Beaumont Hospital. Time to *Tri* to evaluate the North Dublin fungal dermatophyte landscape.

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Abstract

Background

Trichophyton species, a group of arthrophilic dermtophytes, are the leading cause of superficial fungal skin infections in humans. Keratin is required for growth in host tissue, meaning that trichophyton species infect the skin, hair and nails. These infections are highly prevalent globally, transmitted by person-to-person contact, with risk factors such as close contact, moist environment and pre-existing skin conditions. Their prevalence and species distribution in North Dublin has not previously been studied.

Objectives

To undertake a microbiological and epidemiological review of *Trichophyto*n species isolated in Beaumont Hospital focusing on species distribution and clinical significance.

Methods

Positive *Trichophyton* species specimen laboratory results between 2000 and 2020 were extracted from the Beaumont Hospital Information System (BHIS). Data analysis was performed using Microsoft Excel where specimens were categorised according to specimen type and site of sampling.

Results

2193 trichophyton positive specimens from 2112 patients were studied. This included inpatient and outpatient sources. 54.9% of patients were male, 44.7% female and 0.4% unassigned. Positive results were categorized by specimen type (nail clippings: n=1686, skin scrape: n=478, hair: n=9, unspecified: n=14, swabs: n=3, tissue: n=2, skin lesion: n=2). Median age of patients was 45 years. *Trichophyton rubrum* was the most prevalent pathogen (65%: n=1446) followed by *Trichophyton interdigitale* (25.95%: n=569). There was no invasive disease.

Conclusions

Trichophyton species infections represent a significant clinical challenge due to their prevalence, variety of presentation and potential for recurrence. Understanding the epidemiological distribution of these infections is important from a public health perspective to guide effective prevention strategies.

A027

Amid Magic and Menace: Psychiatrists' attitudes to Psilocybin therapy

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Abstract

Background:

Psilocybin is naturally occurring tryptamine alkaloid, with psychoactive properties, produced by numerous fungi. There is preliminary clinical evidence supporting the therapeutic potential of psilocybin, including phase 3 trials for treatment-resistant depression. The approval of psilocybin as a medical treatment for depression in certain jurisdictions underscores the need for a balanced and scientific understanding of psilocybin therapy. A contemporary appreciation of psychiatrists' knowledge and attitudes to the rapidly evolving field of psilocybin therapy is a vital component in this process.

Objectives:

To assess the knowledge and attitudes of (Irish) psychiatrists to the therapeutic use of psilocybin, and to explore factors that influence these attitudes.

Methods:

An anonymised 28-item questionnaire was designed and distributed via an online link. Data were collected using Qualtrics and analysed using SPSS Statistics.

Results:

151 psychiatrists completed the questionnaire. Of these, 82% agreed that psilocybin therapy shows promise in the treatment of psychiatric disorders, ranging from 80% in depression to 9% in psychotic disorders. 15% of the total sample reported at least one concern qualitatively including lack of evidence, cost and accessibility.

Conclusion:

Overall, attitudes to the therapeutic potential of psilocybin therapy were mostly favourable. Younger psychiatrists tended to hold more positive attitudes towards the potential use of psilocybin therapy as a treatment for specific psychiatric disorders and demonstrated a willingness to refer patients if it was an evidence-based, licenced therapy. More senior psychiatrists were less willing for psilocybin with psychological support to be granted medical treatment status, even if supported by evidence from clinical trials.

A028

Insights into yeast dynamics in artisanal stretched-curd cheeses: influence of wooden biofilms, processing surfaces, and seasonality

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Abstract

Yeasts influence artisanal cheeses, affecting both flavour and spoilage. Nevertheless, their ecology, particularly in artisanal stretched-curd systems, remains poorly investigated. For such productions, the acidification process is often performed overnight on wooden tables, where members of the microbial consortia present in biofilms likely contribute to the fermentations. This study investigated two artisanal stretched-curd cheeses, namely Provola dei Nebrodi and Ragusano PDO cheeses, during different seasonal productions: spring-summer and winter, respectively. The impact of microbiota on both acidification and moulding wooden tables was compared with that on steel or plastic tables, respectively. Yeasts were enumerated using culture-based methods, and fungal communities were profiled by ITS amplicon sequencing. In Ragusano cheesemaking, yeasts increased by 2.7–2.9 log CFU g^{-1} after acidification, reaching 5.46 log CFU g^{-1} and 4.55 log CFU g^{-1}, on wood and steel tables, respectively. Conversely, in the Provola dei Nebrodi production, culture-based counts showed no differences. In both cheese production, wooden tables were consistently dominated by *Pichia* spp. and *Saccharomyces cerevisiae*. After acidification, a material-specific selection was observed, with *S. cerevisiae* and *Pichia* enriched on wood with a season-dependent behaviour. Moreover, in wood-moulded cheeses, yeast values remained always higher than in plastic-moulded samples, with an observed divergence: cheeses moulded in wooden tables were richer in *Pichia*, whereas cheeses moulded in plastic tables were richer in *Kluyveromyces lactis*, *Torulaspora quercuum*, *Kazachstania unispora*, and *Candida pseudoglaebosa*. These findings delineate season and material-driven fungal communities and establish a baseline to evaluate their impact on quality, stability, and defect risk in artisanal practice.

Session : Out of Body Experience- in vitro models of Infection (Complex cell infection models)

A029

Developing an animal-free microaerobic human intestinal epithelial model to study host-microbiota interactions in Crohn's disease

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Abstract

Crohn's disease (CD) is a chronic inflammatory bowel condition affecting over 120,000 individuals in the UK. It is marked by debilitating symptoms and increased risks of colorectal cancer and surgical intervention. While current treatments offer symptomatic relief, the underlying causes remain elusive. Emerging evidence indicates that genetic mutations in affected individuals lead to a disrupted crosstalk between the intestinal epithelium and resident microbiota.

This project aims to develop a physiologically relevant animal-free model system to culture human intestinal epithelia with the mostly anaerobic gut microbiota by combining a microaerobic vertical diffusion chamber (VDC) with patient-derived human intestinal organoids.

Our results demonstrate that we can successfully culture a simplified human microbiota (SIHUMIx) in animal-free media in the VDC system. In addition, we have established xeno-free serum alternatives and basement membrane coatings for growing polarized human intestinal epithelia. Current studies are exploring synthetic hydrogels as Matrigel alternatives in human intestinal organoid culture.

Taken together, this project will develop a physiologically relevant platform to study epithelial–microbial crosstalk in CD. This will open avenues for microbiota-based therapies and personalized medicine. Furthermore, it will reduce the reliance on animal models and enhance translation of in vitro research into clinical applications.

A030

Investigating early immune interactions between ASFV and porcine dendritic cells in *ex vivo* and *in vitro* cultures

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Abstract

African swine fever virus (ASFV) induces a lethal haemorrhagic disease in pigs and the ongoing ASF panzootic severely threatens the global pig industry. Understanding host responses to ASFV is challenging due to the complexity of the virus. We previously demonstrated that ASFV disrupts the innate-adaptive immune axis *in vivo* and found that this was associated with reduced dendritic cell (DC) frequencies. Detailed investigations *in vivo* are limited to one virus per host; hence, we sought to investigate strain related ASFV-DC interactions with *ex vivo* spleen derived suspension cultures and *in vitro* bone marrow derived DC (BM-DC) cultures.

Spleen cell suspensions were obtained from inbred Babraham pig spleens and cultured in ultra-low adhesion plates. Bone marrow cells from the same animals were cultured for nine days with human FLT3-ligand to derive BM-DCs. Both cultures were infected with ASFV strains of varying virulence or recombinant ASFV expressing fluorescent proteins and monitored over two days post-infection. Infection was assessed using live imaging (Incucyte) and virus titrations, while flow cytometry was employed for phenotyping and assessing activation status of myeloid cells.

Both spleen and BM-DC cultures were stable and supported ASFV replication. BM-DC cultures were significantly enriched for DCs and included a mixture of other myeloid-derived cells. DCs in both cultures were susceptible to ASFV infection, confirming previous *in vivo* results. DC tropism and activation marker expression profiles differed between strains of ASFV, indicating strain-specific modulation of host immune responses. These cultures provide tractable platforms for deeper investigations into the mechanisms driving these responses.

A031

The development of a kidney organoid model of JC Virus infection

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Abstract

JC virus (JCV) is a polyomavirus that establishes a persistent but asymptomatic infection in the kidneys of 30-50% of healthy individuals. It is a non-enveloped, double stranded DNA virus with a viral genome composed of a noncoding control region, an early gene expression region, and a late gene expression region. In patients with prolong and severely weakened cellular immunity, JCV can reactivate and undergo genomic rearrangement, allowing the virus to infect glial cells of the central nervous system. This can result in the rare and often fatal demyelinating disease Progressive Multifocal Leukoencephalopathy (PML). Currently there are no *in vitro* models that accurately recapitulate the pathological processes of PML. 3D organoids are cell culture models derived from human pluripotent stem cells or adult stem cells and can mimic tissue morphology, cellular heterogeneity, and function of human organs. We have shown successful JCV infection and replication within human kidney organoids through detection of increasing production of viral genome and late viral protein over 21 days. This is the first reported successful infection of JCV in kidney organoids. This model will be used to test the efficacy of various viral replication inhibitors in JCV infection. The development of an *in vitro* model of JCV infection could improve preclinical screening for drug candidates for PML.

Session : Genetics and Genomics Forum

A032

Development of a type II(S) restriction enzyme-based approach to enable specific enrichment of genome-transposon junctions in chlamydial transposon mutant libraries.

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Abstract

Background: *Chlamydia trachomatis* is the most common bacterial sexually transmitted infection in the UK. Formulating a vaccine requires knowledge of gene function. Therefore, a transposon mutagenesis approach to identify gene function has been developed, this works by inducing transposons that were cloned into a shuttle vector, to jump into the *C. trachomatis* genome. Sequencing of the surviving pool of transposon mutants is then used to identify essential genes. However, a large number of undelivered transposons remain after induction, requiring time-consuming work to identify the transposons that have jumped into the chlamydial genome versus those which remain in the vector.

Aims: Use a CspCI type IIS restriction enzyme to fragment vectors which contain undelivered transposons after induction, reducing sequencing of uninduced transposons in the vector and increasing the amplification of true transposon insertions within the chromosome.

Methods: The first step was cloning a CspCI recognition site into the existing p2TK2-RiboA-C9Q transposon delivery vector. The optimal drug concentrations and growth stage for induction were then identified. Induction was then performed, and some samples were digested with the CspCI restriction enzyme. All samples were sequenced, and the resulting data compared.

Results: Cloning of the CspCI recognition site into the vector was successful. Treatment with CspCI reduced the sequencing of un-delivered transposons in the vector, although this result was seen regardless of whether the transposon delivery vector contained the CspCI recognition site.

Conclusion: Further work will be required to identify the significance of these results and improve the process to achieve saturation mutagenesis.

A033

SeroBA and SeroBAnk: A robust genome-based serotyping scheme and comprehensive atlas of capsular diversity in *Streptococcus pneumoniae*

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Abstract

Background

The unprecedented rate of pneumococcal sequencing in recent years has accelerated the discovery of new serotypes; 9 new serotypes have been discovered since 2020. However, current bioinformatics tools are infrequently updated, so cannot identify these new serotypes. We present SeroBA(v2.0) which can identify 102 of 107 serotypes, and the SeroBAnk - a community driven, web based pneumococcal information atlas.

Methods

We built SeroBA(v2.0) upon SeroBA(v1.0.7), integrating serotype-specific genetic information into the SeroBAnk. We validated SeroBA(v2.0) using 26,306 pneumococcal genomes from the Global Pneumococcal Sequencing (GPS) project as well as simulated data for the 102 reference sequences of the capsular encoding region which were simulated using samtools.

Results

Of 17,933 pneumococcal isolates with phenotypically determined serotypes in the GPS project, SeroBA correctly predicted the serotype of 15,945 (89.3%) isolates. SeroBA correctly predicted the serotype of all 102 simulated capsular reference sequences. We also discovered that the newly added serotypes (15D, 20A, 20B, 20C, 33E, 33G, 33H, 36A, 36B) make up 0.4% (106/26,306) of isolates in the GPS project. The SeroBAnk web resource, containing the genetic basis for each serotype is now available (https://www.pneumogen.net/gps/#/serobank).

Conclusion

SeroBA(v2.0) can accurately identify 102/all known pneumococcal serotypes. Some serotypes cannot be resolved as their genetic basis has not been fully defined. In addition, SeroBAnk provides the first community driven comprehensive pneumococcal information atlas to which people can submit novel serotypes.

A034

kan you keep a secret? Genomic position is key to antimicrobial resistance phenotype

Caroline Jarvis ORCID iD[1,2,3], Muhammad Yasir[1,3], Emma Holden[1,3], Eleftheria Trampari[1,3], Gemma Langridge[1,3], Mark Webber[1,2,3]

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Abstract

The location and associated context of genes has been shown to have a substantial effect on bacterial gene expression, with genome plasticity and evolution both influenced by chromosome organisation. The rules governing these relationships are not fully understood, but provide a crucial element in understanding genomic evolution, understanding antimicrobial resistance and the rational design of synthetic biological systems. Here, we present further evidence of the relationship between gene location and gene expression, with a focus on antimicrobial resistance genes and subsequent changes to fitness under antibiotic stress.

To explore the biological implications of antibiotic resistance gene location, we have used high-throughput transposon-directed insertion sequencing (TraDIS-*Xpress*) to assay hundreds of thousands of *E. coli* and *S.* Typhimurium mutants – each containing an identical kanamycin resistance cassette (*kanR*) at a unique genomic location.

Analysis of abundance of each mutant allowed us to estimate the fitness impact of the identical kanamycin resistance cassette positioned in thousands of different loci around the chromosomes of *E. coli* and *S.* Typhimurium. Significantly different degrees of susceptibility to kanamycin were conferred by positioning the cassette at different locations. Results were validated using a smaller panel of independent mutants of both species, and growth kinetics of these mutants correlated with the high-throughput data.

Our data show local factors are important in the expression of resistance with no strong relationship being observed with distance from the origin of replication, or chromosome strand. Understanding how location impacts expression of AMR genes can help explain evolutionary events and improve genotype-phenotype predictions.

A035

Location, location, location: how does genome position affect bacterial gene expression?

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Abstract

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 dynamic micro and macro structures of bacterial chromosomes are major influences on transcription.

Here, we present an optimised method to study how genomic context influences gene expression in bacteria, and we have explored how antibiotic stress affects 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, allows the impact of changes in the environment on transcription to be determined. We have correlated this collection of location dependent expression data with precise psoralen sequencing measurements of chromosome supercoiling, to reveal novel results regarding the effect of antibiotics on supercoiling state. Therefore, our new high-fidelity approach 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 through further research with these methodologies will provide insight into genome evolution, antimicrobial resistance and information that can be applied in synthetic biology.

A036

Distribution and Diversity of Type VI Secretion System Gene Clusters in the Achromobacter Genus

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Abstract

Gram-negative species of Achromobacter are emerging opportunistic pathogens in immunocompromised individuals. Achromobacter species coexist with other bacteria in the environment and airways, especially in individuals with cystic fibrosis (CF). The type VI secretion system (T6SS) is utilised to deploy effectors into neighbouring cells, helping bacteria to establish a niche and avoid immune defences. This study explored the presence and diversity of T6SS gene clusters in Achromobacter species.

Complete Achromobacter genomes, from the NCBI database, were investigated using a bioinformatic analysis pipeline. The genomes were first examined using the Type Strain Genome Server and MASH to identify and remove duplicates, and determine individual taxonomy, giving a working dataset of 38 genomes. Next, SecReT6 was used to identify T6SS gene clusters and classify T6SS subtypes based on the phylogenetic analysis of the conserved TssB protein. Cblaster was subsequently employed to define the boundaries of each cluster and generate cluster diagrams for visual comparisons across subtypes.

Our results revealed that the T6SS is widespread across Achromobacter species. In some cases, three different T6SS gene clusters were found per genome. The T6SS i4b subtype was the most prevalent, followed by the i1 and i3 types. Only genomes from A. insolitus carried the i4a type. By separating the Achromobacter strains by isolate source, we found that most A. xylosoxidans isolates, commonly associated with CF, contained only the i4b type. As the first systematic study to characterise the T6SS in Achromobacter genus, it provides a foundation to further explore the role of T6SS during Achromobacter infection.

A037

Battling Biofilms: Investigating the role of *maoP* in Enterobacteriaceae biofilm formation

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Abstract

Biofilm formation is a key process in the life cycle of many important bacteria, where organisms form multicellular aggregates, often stuck to a surface. Despite substantial research into the complex and tightly regulated pathways involved in biofilm formation, we do not have a full understanding of how biofilm formation is regulated. Modifying biofilm formation is an important goal in preventing infection and in biotechnology, but the knowledge base to enable this remains incomplete.

MaoP is a protein originally proposed to be involved in chromosomal organisation, although other work has also identified MaoP as an RNA-binding protein. We have recently shown it to be important in biofilm formation by the key foodborne pathogens *Escherichia coli* and *Salmonella* Typhimurium.

This project aims to characterise how MaoP impacts biofilm formation in Enterobacteriaceae, using *Salmonella*Typhimurium as a model. Here we show that deletion of *maoP* reduces production of the key biofilm components curli and cellulose, due to reduced transcription of the master biofilm regulator *csgD*. We also show that the biofilm deficient phenotype can be recovered in mutants isolated from a biofilm evolution model, with early results being presented here. Finally, we present work towards uncovering RNA species to which MaoP binds using RNA immunoprecipitation sequencing (RIP-seq).

A038

pyani-plus: A Multitool for Average Nucleotide Identity estimation

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Abstract

Overall genome relatedness index (OGRI) methods such as digital DNA-DNA hybridisation (dDDH) and average nucleotide identity (ANI), and genome identity estimates using k-mer based approaches such as Mash, fastANI, and sourmash, are central to taxonomic assignment and classification of microbes in modern microbiology.

The original, widely used pyani software was written to make several ANI methods available in a single bioinformatics tool, parallelising comparisons across multicore machines and compute clusters for rapid analysis of very large microbial genome datasets. We present pyani-plus (https://pyani-plus.github.io/pyani-plus-docs/), completely rewritten from the ground up as part of the genomeRxiv project (https://genomerxiv.cs.vt.edu) to extend and improve the capabilities of pyani.

The new pyani-plus software now supports additional ANI estimation methods, including fastANI and sourmash. ANI calculations are stored in a persistent, shareable local database. The database records software and comparison parameters, and allows for inclusion of new genomes to existing analyses and regeneration of result sets with no need for recalculation, enhancing reproducibility and supporting open research.

pyani-plus supports a wider range of job schedulers for deployment on compute clusters, and is able to resume interrupted analysis runs. A new graph-based hierarchical classification algorithm is implemented that gathers genomes into self-consistent cliques on the basis of ANI value, as an aid to classification.

These new capabilities are wrapped in a friendlier user interface with improved and extended options for graphical output and data exchange.

A039

The Power of Three: Genomic, Gene and Phage Level Variation of Shiga Toxin Subtype *stx2a* in *E. coli* from England, 2016 - 2024

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Abstract

Background: Phage-encoded Shiga toxins (Stx), the hallmark feature of Shiga toxin-producing *Escherichia coli* (STEC), are linked to varying clinical severity. Stx subtype 2a (Stx2a) is associated with a higher risk of progression from foodborne gastroenteritis to severe haemolytic uraemic syndrome (HUS).

Methods: 19,429 *E. coli* genomes submitted to UKHSA between 2016-2024 were extracted from EnteroBase, with serotype and *stx* gene information per isolate obtained from UKHSA and additional *stx* subtyping performed using StxTyper. Serotype distributions were quantified in R and visualised using Grapetree. Gene-level variation was identified using snp-sites, quantified with VCFtools and phylogenetic relationships inferred using IQ-Tree. Prophage regions were extracted using PHASTER and annotated using Pharokka.

Results: Overall, *stx2a* was present in 40.0% (n=4114) of STEC isolates across 76 O serogroups, most commonly in O157 (n = 2274, 17.7%), O26 (n = 824, 6.4%) and O145 (n= 562, 4.4%), with increasing prevalence over time in unique non-O157 serogroups. *stx2a* was most commonly observed alone, in 18.4% (n=2375) of isolates, and was also present in 16 additional *stx* subtype combinations. Gene level analysis identified 89 SNPs with multiple allelic variants clustering by serotype, with additional diversity in further *stx* presence alongside *stx2a*. Phage-level analyses integrated with gene level and genomic context data demonstrate connections between serotype and *stx2a* variants.

Conclusion: *stx2a* is prevalent within UKHSA STEC isolates, with multiple allelic variants and increasing acquisition amongst O serogroups, highlighting *stx2a* as an emerging public health concern. The study also emphasises the value of genomic surveillance within public health.

A040

Till Death Do Us Part: Understanding Tailocin Killing and Binding

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Abstract

Tailocins are a type of phage tail-like bacteriocins produced by bacteria to kill competing bacteria. This research focuses on *P. syringae pv. aptata (Ptt)*, the tailocin derived in the Balturs Labs from Pseudomonas syringae. Previous research in the Baltrus Lab has found that Ptt has produced two types of bacteriocins (F- and R-type) that kill different strains and allow *Ptt* to have a broad range of intra-species targets. The goal of this research is to determine whether the bacterial genome produces anything else that is unaccounted for in the overlay plate. The central question is: "Does Pseudomonas produce other proteins or materials that affect it?" Ptt could be producing another antimicrobial compound that is killing, while there are also tailocins. The primary hypothesis is that there is another phage or byproduct of the bacteria that is impacting the size of the killing zones. If the target radius of the bacteria does not change when the tailocins' ability to function is hindered (through the Ptt Δsheath), then it can be inferred that there is something else being produced that influences the target radius.

A secondary hypothesis is that the bacterial genome of *Pseudomonas* affects the efficiency of tailocin killing. In exploring this possibility, we can determine whether the specific strain of tailocin is more efficient at killing because of an unnoticed anomaly or if more testing needs to be done.

A041

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

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Abstract

Understanding the evolution of antimicrobial resistance (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 host-mimicking media.

Pseudomonas aeruginosa PA14 was selected for resistance to meropenem in caMHB, synthetic wound fluid (SWF) and synthetic cystic fibrosis sputum (SCFM) using an evolutionary ramp approach. Evolved clones were whole genome sequenced and mutations identified bioinformatically and collateral susceptibility to other antibiotics and comparative fitness were assessed.

The maximum meropenem resistance achieved was distinct for each medium. All media promoted increased ceftriaxone and levofloxacin resistance, but only caMHB and SCFM clones showed increased amikacin susceptibility. Following sequencing of selected clones, we identified that only 20 mutations, which were predicted to affect protein function, arose in more than one clone. Only 2 mutations were present in at least one clone selected in the three different media. Several mutations identified have not previously been linked to meropenem resistance.

We have demonstrated that the choice of media to assess AMR in vitro can impact the mutations which arise, collateral networks 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.

A042

Hidden in the Prophage: Genomic Drivers of Persistent *Staphylococcus aureus* Infections

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Abstract

Staphylococcus aureus bloodstream infections remain a major clinical concern due to their high morbidity and mortality. At Parc Taulí University Hospital, we conducted a large-scale genomic study of 339 *S. aureus* isolates from bacteraemia cases collected between 2014 and 2022. The cohort showed 27.4% mortality, 26.3% sepsis and 24.2% embolic complications. Persistence, defined as positive blood cultures three days after effective treatment, occurred in 19.5% of cases and was linked to sepsis and prolonged hospitalization. Dominant clonal complexes included CC30, CC5 and CC45, while the zoonotic, multidrug-resistant ST398 lineage rose from non-existing to 15% over the study period. In the collection, we identified 369 distinct prophage clusters, several of them carrying virulence genes such as Panton-Valentine leucocidin and enterotoxin A, though none harboured antimicrobial resistance genes. We explored the role of prophage genes in infection outcomes, by integrating genomic and clinical data. As a result, two prophage-encoded genes were significantly associated with persistent bacteraemia ($p = 0.029$). These genes were located outside canonical phage modules, adjacent to holin and endolysin genes. Bioinformatic analysis revealed a *hipA*-like kinase domain in one of these genes, resembling the HipAB toxin-antitoxin system known to mediate antibiotic persistence *in vitro* in *Escherichia coli*. HipAB-prophage mobility was demonstrated by induction with mitomycin C and transference to a recipient strain. Our findings suggest a novel prophage-encoded toxin-antitoxin system that may contribute to persistent *S. aureus* bacteraemia, offering new insights into pathogenesis and potential therapeutic targets.

A043

Genome structure determines the pathobiology of multidrug resistant *Acinetobacter baumannii*

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Abstract

To fit in the bacterial cell, the chromosome is folded in a highly organised structure, which ensures the correct execution of processes such as replication, segregation or transcription. The DNA-DNA contacts maintaining the chromosome structure are mediated by structural constrains imposed by highly transcribed regions and nucleoid-associated proteins (NAPs). These NAPs play a dual role in chromosome organisation, acting as DNA scaffolds and regulating the transcription, with both aspects ultimately impacting chromosome structure. For example, the heterodimeric NAP IHF (IhfA+IhfB) mediates sharp DNA turns that allow complex formation between promoter regions, regulators and the RNA polymerase, which may determine phenotypes. Using the critical-priority pathogen *Acinetobacter baumannii* AB5075 as a model, we show the role of IHF in the regulation of virulence-associated behaviours. An AB5075 *ihfA* mutant is impaired for key virulence factors such as biofilm formation, twitching motility and desiccation tolerance, and its virulence is abrogated in the *Galleria mellonella* infection model. Differential RNA sequencing comparing the *ihfA* mutant to the parental AB5075 showed that IHF impacts the expression of 760 genes, including those involved in the phenotypes tested. A high-throughput structural analysis through Hi-C comparing the *ihfA* mutant to the wild-type revealed that short-range DNA-DNA interactions are enhanced across the chromosome in the absence of IhfA, suggesting an impact in the overall chromosome structure of this pathogen. Altogether, our findings demonstrate that IHF mediates the correct DNA folding, thus contributing to the overall native chromosome structure, and critically impacts the regulation of virulence-associates behaviours in *A. baumannii*.

A044

Evasion of Restriction Modification Systems is Key to Unlock the Antibiotic Potential of Entomopathogenic Bacteria

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Abstract

Entomopathogenic bacteria have long been seen as promising candidates for bio-pesticides and sources of novel antibiotics. However, these insect-infecting bacteria often possess robust restriction modification (RM) systems, making plasmid transfer inefficient and genetic engineering difficult. RM systems are ubiquitous bacterial defence systems which recognise and cleave xenogenic DNA. Differentiation between self and non-self DNA is facilitated by two enzymes: a restriction endonuclease (REase) and a modification methyltransferase (MTase). REases recognise specific DNA motifs and cleave those which are inappropriately methylated, and therefore non-self. These same motifs across the host genome are marked as self by MTase methylation, protecting them from degradation. Although important for bacterial survival, these RM systems are responsible for genetic intractability in many species, serving as barriers to horizontal gene transfer.

To overcome the genetic engineering barrier posed by RM systems in the entomopathogenic species *Photorhabdus khanii*, we used two approaches to adapt a transposon mutagenesis plasmid to evade host RM systems. Firstly, we bioinformatically identified potential targets using the rmsFinder pipeline as well as previously identified methylation targets in related species *Photorhabdus luminescens*. Secondly, we used Oxford nanopore sequencing to identify all modified bases in the *P. khanii* genome during growth in standard laboratory conditions. This enabled identification of the methylome under specific conditions and comparison to the bioinformatic approach. RM recognition motifs were then removed from plasmids while retaining amino acid coding, binding sites, and structural features of the plasmid before testing the effect of these changes on our transposon mutagenesis approach.

A045

BvgAS regulation in Bordetella hinzii and Bordetella avium

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Abstract

The Gram-negative bacteria *Bordetella avium* and *Bordetella hinzii* are primarily responsible for respiratory infections in birds. *B. avium* causes turkey coryza therefore exemplifying the economic significance of this disease within the poultry industry. In addition, there have been reports of human infections with these species, mainly in immunocompromised individuals. However, *B. hinzii* is now considered to be an emerging zoonotic human pathogen highlighting the importance of comprehending both species' mechanisms of pathogenicity and choice of hosts.

The 'classical' *Bordetella* species include *B. pertussis* (the causative agent of whooping cough), *B. bronchispetica* as well as *B. parapertussis.* Each possess differering host preferences while sharing many virulence factors although some are unique to their species. Bordetella virulence gene expression is mainly governed by the master two-component BvgAS regulatory system. This system controls three phenotypic phases: bvg^+, bvg^i (intermediate), and bvg^- which are dependent on the intracellular concentration of phosphorylated BvgA, controlled by the histidine sensor kinase BvgS. Here we aim to compare the differences in transcriptional expression patterns of genes between the bvg^+ and bvg^- phases in *B.avium* and *B. hinzii* using RT-qPCR and RNA-seq. These transcriptome profiles will grant us a deeper understanding of the adaptation of these species to their hosts as well as their pathogenicity in comparison to the other *Bordetella* species.

A046

The impact of particulate pollutants on host-pathogen-commensal interaction

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Abstract

Particulate matter (PM) is a key component of air pollution that negatively impacts both the respiratory health of a host alongside their lung microbiome. Exposure to PM can cause physical damage to the respiratory system, making a potential host more susceptible to infection by pathogens such as *Haemophilus influenzae* and *Moraxella catarrhalis.* The lung microbiome is a collection of naturally occurring microbes, including commensal bacteria, such as *Streptococcus mitis* and *Rothia mucilaginosa,* which rarely cause infection and occasionally have protective effects on the host against pathogens. Although a lot of work has been done on the direct impact of PM on the host, little is known about its effect on bacteria. My work includes observing the impact of black carbon (BC), a model of PM, on both pathogens (*H. influenzae* and *M. catarrhalis),* and commensals (*S. mitis* and *R. mucilaginosa* and *Prevotella melaninogenica).* Pathogen work includes observing how bacteria's growth, adherence to human cells and biofilm formation, as well as specific genes involved in these factors and others, such as the iron-starvation response in *H. influenzae*, which may be affected by BC.

A047

Creating a reference-free molecular epidemiology framework for *Helicobacter pylori*

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Abstract

Helicobacter pylori is a chronic gastric pathogen that infects around half of the world's population, often leading to gastritis, ulceration and occasionally cancer. The genome of this pathogen is small, yet highly recombinogenic and genetically diverse, making comparative genomics analysis difficult, e.g. genome assembly or reference mapping approaches for calling single nucleotide polymorphisms (SNPs). Such tasks are core for typing strains and detecting transmission clusters. Current typing methods rely on a reference genome and a small set of genes such as those for multi-locus sequence typing (MLST), accessory gene content, repeat regions in the *cagA* gene, or polymorphisms in the *vacA* gene. To date, there is no typing approach which utilises the entire genome to differentiate strains, as is common in many other bacterial pathogens.

This project aims to address this challenge by estimating the effectiveness of a reference-free distance estimation (using SKA-2) to approximate the SNP distances between clinical isolates. We use a set of over 1,000 complete genomes to first estimate the precision of SKA2 for calculating SNP distances compared to a ground truth estimated by DNAdiff whole genome alignments. Building on this correlation data we then compare the clustering of isolates based on the SKA2 distances to existing typing methods such as MLST, and *cagA* motifs.

Our findings indicate that reference-free SNP distance estimate may provide reliable estimates of SNP distances within *H. pylori* and allow for rapid transmission and strain typing analysis of this pathogen with low computational resources.

A048

Species-specific genetic targets of single-step ciprofloxacin resistance-conferring mutations influence collateral susceptibility

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Abstract

Mutations in DNA gyrase and efflux pump-mediated extrusion of the drug are predominant mechanisms that confer resistance to fluoroquinolones in Gram-negative bacteria. However, little is known about the effect of genetic background on single-step mutations these bacteria acquire to develop resistance to fluoroquinolones. In this study, single-step spontaneous mutants with decreased susceptibility to ciprofloxacin were isolated from multiple clinical strains of *Escherichia coli* and *Klebsiella pneumoniae*. Whole-genome sequencing of the mutants revealed that in three out of four strains of *K. pneumoniae,* ciprofloxacin-selected mutations were present in the transcriptional repressor OqxR of the OqxAB efflux pump, while the fourth strain acquired a mutation in the transcriptional repressor RamR that regulates the AcrAB efflux pump. Despite AcrAB being one of the major efflux pumps in *E. coli,* all the ciprofloxacin-selected *E. coli* mutants instead developed mutations in DNA gyrase subunit A. In addition, mutants isolated from *K. pneumoniae* showed resistance to trimethoprim, nitrofurantoin and cefalexin; however, this collateral resistance was not present in any of the *E. coli* mutants. Furthermore, the overexpression of AcrAB pump in *E. coli*, achieved by knocking out its repressor-encoding gene *acrR*, was not found to be associated with a decrease in ciprofloxacin susceptibility, fitness costs or collateral resistance to the antibiotics tested. These results suggest that antibiotic resistance-conferring mutations arise differently at both strain and species levels. This study also highlights that the design of effective treatment strategies may depend on the genetic background in which antibiotic resistance-associated mutations develop.

A049

Understanding the specialised metabolite diversity of *Burkholderia gladioli* strains associated with health and rotten onions

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Abstract

Burkholderia species are environmental Gram-negative bacteria with multiple beneficial roles in agriculture, but also harmful traits for human health. While certain *Burkholderia* promote plant growth and protect against crop pathogens, their use as commercial biopesticides declined after *Burkholderia* emerged as problematic lung infections in people with cystic fibrosis in the 1990s. However, due to growing concerns over toxic chemical pesticides, interest in *Burkholderia*-based biopesticides is resurging as natural and sustainable agricultural solutions.

Previous work found that polyyne specialised metabolites such as cepacin A and caryoynencin are capable of protecting peas from the damping-off-disease-causing oomycete *Globosporangium ultimum* (*Pythium ultimum*). This work aimed to further identify the beneficial properties of *Burkholderia,* focusing on *B. gladioli* as novel antibiotic producers and contrasting this with their negative trait of causing onion rot.

A collection of 36 *B. gladioli* isolates recovered from healthy and rotten onions was assembled and screened for antimicrobial metabolite production using microbial antagonism overlay assays and HPLC analysis. Onion isolates were broadly bioactive, with all strains showing antibacterial and antifungal activity. Key metabolites identified in the onion strains were toxoflavin and caryoynencin. Deletion of toxoflavin and caryoynencin reduced antimicrobial activity, but did not remove it, indicating other bioactive metabolites remain. Rot assays demonstrated that deletion of toxoflavin reduced *B. gladioli*'s ability to rot onions, indicating this could be a key metabolite in its plant-pathogenic nature.

In summary, onion-associated *B. gladioli* can produce a wide range of bioactive specialised metabolites and their potential as natural pesticides needs balancing against their pathogenic ability.

A050

Identifying Novel Gene Functions in *Vibrio Cholerae* C6706 Using Chemical Genomics Approaches

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Abstract

Vibrio cholerae is a gram-negative bacterium which causes the disease Cholera, of which there are up to an estimated 4 million cases a year, with 140,000 of these being fatal (WHO, 2024). Most outbreaks occur in developing nations or areas hit by natural disasters, where sanitation infrastructure is insufficient. Transmission of cholera occurs via the faecal-oral route, and disease symptoms include profuse watery diarrhoea and vomiting, which can lead to coma and death in serious cases. The El Tor biotype of V. cholerae is predominant and is responsible for the 7th ongoing cholera pandemic. The organism has a biphasic lifecycle, which includes a vast marine reservoir where the organism persists on chitinous surfaces within biofilms.

Despite extensive research on the role of many key genes involved in switching between these two lifestyles and an abundance of genome sequence information, a large portion of many published V. cholerae genomes consists of unannotated genes. Hence, research into developing targeted interventions is hindered.

To find functions for unannotated genes, we used a chemical genomics approach to phenotypically profile a V. cholerae C6706 single gene mutant library in 91 unique conditions. We were able to identify phenotypes for 1670 of the 3040 mutants screened. Strikingly, 52% of these phenotypes were identified for mutants in unannotated genes. Here we present the results of the screen and some interesting examples from the dataset, together with how the scientific community can access and use the data as a resource.

A051

The pQBR mercury resistance plasmids: a model set of sympatric environmental mobile genetic elements

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Abstract

Plasmids are extrachromosomal elements that can facilitate rapid bacterial adaptation by transferring genes between individuals. Conceptually, plasmid genomes can be split into conserved 'backbones', encoding genes for plasmid functions, e.g. replication, and diverse and flexible accessory genomes, often carrying traits that alter the fitness of host bacteria, thus plasmid fitness. These accessory traits are often encoded on transposable elements, e.g. transposons, able to mobilise between chromosomes and plasmids, enabling traits to spread to new genomes via the plasmid. Most of what we know about plasmids comes from antimicrobial resistance (AMR) plasmids isolated after the widespread introduction of antibiotics in healthcare and agriculture. Selection for resistance can shape plasmid evolution, and AMR plasmids have been shown to share 'backbones' with plasmids lacking resistance genes, suggesting ancient lineages. The pQBR collection is a set of sympatric plasmids isolated from the phytosphere of sugar beets and has been extensively used to expand our knowledge of plasmid evolutionary ecology in the last 30 years. Several pQBR plasmids have been sequenced previously, however, previous studies focused on specific genes or individual divergent representatives. Here we increase the repertoire of sequenced pQBR plasmids, which fall into distinct groups with intragroup differences driven by recent, prolific transposon insertions. Backbone regions of each group contain numerous genes, involved in functions beyond replication and conjugation, including for chemotaxis and radical SAM enzymes, and divergent plasmid relatives include multi-drug-resistant plasmids. Using a novel assay, we also show that the pQBR plasmids vary widely in their capacity for mobilising chromosomal transposons.

A052

Interspecies interaction between *Burkholderia cenocepacia* and *Staphylococcus aureus* in a Cystic Fibrosis–Like Environment

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Abstract

Many bacterial infections are polymicrobial, characterised by the presence of multiple co-existing bacterial species. In people with cystic fibrosis (pwCF), polymicrobial infections commonly involve *Staphylococcus aureus* and *Pseudomonas aeruginosa*, whose interactions are well characterised. In contrast, the relationship between *S. aureus* and *Burkholderia cenocepacia*, another important CF pathogen, remains poorly understood.

To investigate how *B. cenocepacia* influences *S. aureus*, we used a controlled exposure approach using cell-free exoproducts from *B. cenocepacia* cultures. After optimisation in nutrient-rich media, experiments were performed in Synthetic Cystic Fibrosis Sputum Medium 2 (SCFM2) to better mimic the CF lung environment.

We discovered that exposure to *B. cenocepacia* exoproducts elicited broad transcriptional changes in S. aureus USA300, affecting pathways linked to virulence, metabolic reprogramming, and stress responses. Several differentially expressed genes indicated a shift toward a more persistence-like physiological state. Some of the differentially expressed pathways mirror transcriptional profiles previously linked to β-lactam tolerance, suggesting that *B. cenocepacia* exoproducts may prime *S. aureus* for reduced antibiotic susceptibility.

These findings demonstrate that *B. cenocepacia* exoproducts reprogramme *S. aureus* physiology under cystic fibrosis–like conditions, promoting transcriptional patterns consistent with persistence and antibiotic tolerance.

Future work will assess responses in clinical CF *S. aureus* isolates, and determine how these interactions influence antibiotic tolerance and virulence

A053

High-Throughput Chemical Genetic Screening of *Acinetobacter baumannii*

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Abstract

Abstract

Acinetobacter baumannii is a hospital-acquired pathogen with extensive antimicrobial resistance and is recognised by the World Health Organization (WHO) as a critical priority organism. Many of its genes remain poorly understood, limiting insights into stress responses and resistance mechanisms. High-throughput chemical-genetic screening was conducted on an ordered library of ~4,000 mutants across 100 diverse chemical and environmental stress conditions. Automated imaging and analysis tools, including IRIS and ChemGAPP, were used to assess fitness and colony morphology. This approach allowed the identification of stress specific phenotypes and the uncovering of shared patterns among genes. The study provides a functional overview of *A. baumannii* and a valuable resource for discovering resistance associated genes and potential therapeutic targets.

A054

Phase variation of lipooligosaccharide-modifying genes shapes disease-associated phenotypes in nontypeable *Haemophilus influenzae*

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Abstract

Non-typeable Haemophilus influenzae (NTHi) are both commensals and major human pathogens, causing infections of the middle ear, lower respiratory tract, and more recently invasive infections. Their high adaptability is driven in part by phase variation (PV) where slipped-strand mispairing modulates expression of key surface structures, particularly the lipooligosaccharide (LOS).

LOS composition influences virulence and immunoevasion, complicating treatment and vaccine design. This study investigates how PV architectures in LOS biosynthesis genes vary across different clinical contexts and explores their contribution to niche-specific adaptation. A curated dataset of 2,972 public NTHi genomes was analysed to quantify PV in seven LOS-biosynthesis loci (*lic1A, lic2A, licA/B, IgtC, lex2A*, and *oafA*). Simple sequence repeat (SSR) tract lengths were quantified and stratified by disease metadata and significant differences in SSR lengths were observed between disease groups. COPD-associated isolates exhibited longer SSRs in most genes, particularly in *licA, lic2A* and*IgtC*, consistent with increased switching frequency and potential ON-state bias supporting mucosal persistence and biofilm formation under chronic inflammation pressures. Alternatively, invasive isolates display shorter, stabilised SSRs, especially in *licA, lic3A* and *lex2A* with predicted OFF-expression that reduces complement activation and serum killing. Mucosal isolates instead show heterogenous distributions that may reflect fluctuating immune pressures during mucosal spread. Together, these findings show that disease niches select for SSR tract lengths that select different reading frames and therefore expression states for distinct LOS structures.

Understanding these dynamics will improve prediction of disease, support the development of PV-aware diagnostics and identify particular PV targets for intervention.

A056

Bacterial chromatin structure and genetic recombination along the *Streptomyces* chromosome

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Abstract

Streptomyces are rhizospheric bacteria involved in geochemical cycles. Their complex developmental cycle is associated with the production of specialized metabolites exploited in biotechnologies. Bacteria of the *Streptomyces* genus have a large linear chromosome, up to 12 megabases, subjected to high genetic instability associated with large chromosomal rearrangements (deletions, amplifications, translocations, circularizations). In the laboratory, it has been shown that the repair of DNA double-strand breaks is responsible for large-scale genomic rearrangements, triggering homologous or illegitimate recombination events (Hoff *et al*., 2018). These rearrangements preferentially affect the terminal regions of the chromosome, called chromosomal arms, in which many specialized metabolite biosynthesis gene clusters are found. In *Streptomyces ambofaciens*, this compartmentalization is correlated with (i) evolutionary pattern: the chromosome arms evolving faster than the central part (Lorenzi *et al*., 2021) and (ii) spatial structuring in vivo: the central region is more strongly partitioned into multiple chromosome interaction domains, while the terminal regions are enriched in mid-range contacts (Lioy *et al*., 2021). The working hypothesis is that the spatio-temporal dynamic of chromatin is at the origin of the evolutionary compartmentalization of the *Streptomyces* chromosome. Our project aims to explore the link between chromatin composition (DNA-associated proteins: NAPs and SMC), and molecular recombination processes in *S. ambofaciens*. Two axes are explored: (i) the chromosome accessibility and the distribution of the NAP HupS, for which we observed a dynamic relocation to the terminal regions (ii) the balance between homologous and illegitimate recombination along the chromosome.

A057

Predicting novel blaKPC-2 mutations conferring resistance to ceftazidime-avibactam: Implications for genomic surveillance and clinical diagnostics.

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Abstract

The β-lactam/β-lactamase inhibitor combinations (BLIC), ceftazidime-avibactam (CZA) and meropenem-vaborbactam (MEV) are widely used to treat infections caused by KPC-producing Enterobacterales. However, resistance to BLICs is rising, often mediated by mutations in β-lactamase genes that disrupt enzyme-inhibitor interactions. Currently, genomic surveillance fails to detect uncharacterized inhibitor-resistant mutants, leading to genotype-phenotype discordance and limiting effective antibiotic selection.

We aim to identify novel mutations in bla_{KPC-2} that confer resistance to CZA and MEV using a molecular approach that overcomes constraints of in vitro evolution.

A mutant library of bla_{KPC-2} was generated via random mutagenesis, cloned into a low-copy plasmid, and screened for resistance to CZA and MEV. 2× Minimum Inhibitory Concentration (MIC), 4×MIC and clinical breakpoint were tested. Short-read sequencing of plasmid populations was used to identify single nucleotide polymorphisms (SNPs). Mutations were mapped to the wild-type bla_{KPC-2} and assessed using SIFT (Sorting-Intolerant-From-Tolerant) to predict effects on enzyme function.

No SNPs conferred resistance to MEV. Diversity of SNPs conferring resistance to CZA was inversely proportional to CZA concentration. SNPs clustered in three regions commonly associated with CZA resistance: β3–β4 loop, β5–α11 loop, and omega loop. Identified mutations such as A171, D178, V239 and T242 matched those found in resistant clinical isolates. Thirty-nine (~31%) non-synonymous mutations were predicted to affect enzyme function.

We identified a substantial reservoir of uncharacterized bla_{KPC-2} mutations that confer CZA resistance. These findings have immediate implications for genomic surveillance, diagnostics and antimicrobial stewardship, as incorporation of these novel variants into resistance databases will enhance molecular diagnostics.

A058

From Carriage to Disease: Characterising a Transition State Using Genotype-Phenotype Correlation Rules in Neisseria meningitidis

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Abstract

Background: Understanding the molecular mechanisms underlying the transition of *Neisseria meningitidis* from carriage to invasive disease remains a critical challenge. We aimed to identify the transition mechanisms mediating the switch from health to disease of hyperinvasive meningococcal isolates. Ultimately, we aimed to develop a computer model recapitulating this mechanism to test potential interventions *in silico*. **Methods:** Existing genotype (phase variation ON and OFF states) and phenotype data from 163 N. meningitidis isolates were analysed. Unsupervised hierarchical k-means clustering (HK) was used to identify distinct clusters, and statistical tests (Wilcoxon rank-sum and Fisher's exact) assessed associations between gene states and phenotypic traits. Genotype–phenotype rules were extracted to capture key mechanistic relationships. All analyses were conducted using R. **Results:** HK-means clustering identified three distinct adaptive states: carriage (C1), transition (C2), and disease (C3). The C1 state exhibited high serum resistance, biofilm formation, and adhesion, whereas the C3 state displayed high growth rates in BHI media alongside reduced adhesion and growth rates in minimal media. The transition cluster (C2) exhibited selective attenuation, with reduced adhesion, serum survival, and altered nutritional growth characteristics, representing an intermediate adaptive phenotype. The transition state isolates were genetically characterised by differential expression states for seven phase-variable genes (e.g. *pilC1* ON, *hmbR* ON),. **Conclusion**: We identified a distinct transition state bridging carriage and disease, defined by a hybrid genetic and phenotypic signature. The extracted genotype–phenotype rules provide a mechanistic framework for agent-based modelling, enabling simulation of meningococcal adaptation and prediction of progression from carriage to invasive disease.

A059

Improved post-enrichment metagenomics to capture within-sample *E. coli* diversity at sub-lineage resolution

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Abstract

Understanding the transmission of clinically relevant drug-resistant pathogens, such as *Escherichia coli*, is complex but crucial for effective infection control. Current surveillance methods typically fail to capture the within-species diversity of samples, which is essential for linking transmission between different sources. Emerging metagenomic approaches hold promise in capturing this variation and can computationally assign sequencing reads to specific lineages, but the most appropriate method remains unclear.

We evaluated metagenomic methods to assess their performance in capturing the diversity of *E. coli* at sub-lineage resolution and detecting low-abundance variants in complex samples. To achieve this, we created *in vitro* models of stool and sewage, spiked with proportion-controlled mixtures of five *E. coli* lineages spanning a range of single nucleotide variant (SNV) distances (from narrow (clades within a sequence type) to wide (different phylogroups)). We sequenced these samples using shotgun metagenomics from directly extracted DNA, and culture-based post-enrichment metagenomics, whereby we enriched samples in MacConkey media, in broth and on agar, before extracting DNA. Post-enrichment outperformed shotgun metagenomics by increasing the percentage of sequencing reads mapping to *E. coli* from <1% to 95-98% in spiked stool models and improving lineage resolution.

We show that post-enrichment metagenomics is a promising tool to increase resolution for pathogen surveillance and likely provides a cost-effective method to improve the detection of low-abundance lineages from complex samples. We will next test the applicability of these workflows, alongside further optimising bioinformatic pipelines for lineage-resolution, using samples from different One Health compartments and settings in Africa and Europe.

A060

Genomic epidemiology of *E. coli*, plasmids & AMR in the Malawian poultry network and clinic: A One health approach

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Abstract

Understanding the complex epidemiology of WHO-priority pathogens, such as *E. coli*, and their antibiotic resistance (AMR) patterns necessitates a holistic One Health approach. The interactions between bacterial hosts, environmental factors in agricultural settings, and clinical settings can lead to the spread and coalescence of antimicrobial resistance genes. This study generated 290 nanopore sequenced *E. coli* strains, characterising a 60-year-old poultry supply network from breeder to smallholders. Bacterial sharing events were identified at ecological interfaces and documented persistence through the network at several transmission thresholds. In addition, the study examined the network's relationship with clinical settings by comparisons to 699 Malawian *E. coli* sequences. The findings on plasmid epidemiology revealed concurrence alongside new dissemination pathways, distinct from strain-level dynamics, highlighting additional complexities and the role of plasmids in AMR carriage. The implications for AMR spread indicated that, while agricultural networks may not present frequent risks, they can establish pathways that enhance AMR persistence on farms. This research underscores the importance of integrated surveillance across ecological compartments to mitigate AMR threats effectively.

A061

Metagenomic examination of the relationship between prophage presence and infection in Enterococcus faecalis.

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Abstract

Horizontal gene transfer is a key driver of bacterial genome evolution, often mediated by temperate bacteriophages. These viruses integrate into host chromosomes and form prophages, which can encode lysogeny regulators, defence mechanisms and virulence factors. While prophage-associated virulence and resistance to other bacteriophages has been well-characterised in other Gram-positive opportunistic pathogens such as *Staphylococcus aureus*, their role in *Enterococcus* species remains underexplored. This study investigates the relationships between prophage presence, infection site, and clinical outcome in *E. faecalis*, assessing the variation between integration sites and accessory genes in the context of the *E. faecalis* population structure. A large study using whole-genome sequence comparisons has shown a correlation between prophage3 and clinical isolates compared to carriage (doi: 10.1128/spectrum.00201-23). Building on these findings, we will investigate whether the association of prophage 3 with clinical isolates persists across a broader *E. faecalis* population by analysing publicly available genomic data. We will compare prophage3 integration sites, sequence variation, and gene content, explore associations with isolate metadata and evolutionary dynamics underlying these patterns.

A062

The Virulence Plasmid of a Food-Borne *Cronobacter sakazakii* strain MK_1 Encodes a Contact-Dependent Growth Inhibition (CDI) System

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Abstract

The vast majority of bacteria of the genus *Cronobacter* carry closely related virulence plasmids (pVirCro). Genome analysis of the food-borne isolate *C. sakazakii* MK_1 revealed that this strain carries a contact-dependent growth inhibition (CDI) locus, not previously characterized in *Cronobacter*. These systems produce polymorphic toxins (CdiA) and cognate immunity proteins (CdiI) that mediate interbacterial competition *via* direct cell contact. This study aimed to investigate the structure and activity of the CDI-related locus of strain MK_1 and analyse the distribution and diversity of similar loci among bacteria of the genus *Cronobacter*. Genome of *C. sakazakii* MK--_1 was sequenced using Oxford Nanopore and Illumina, annotated with Bakta and manually corrected. Protein features were predicted using HMMSCAN, INTERPROSCAN, and AlphaFold. Alignments were generated using MAFFT and Biopython. Promoters were predicted with BPROM and experimentally validated. Phylogenetics were conducted using PSI-BLAST, IQ-TREE. The MK_1 genome comprises a circular chromosome and three plasmids, including the pVirCro-family replicon pCS-MK1_P1, which encodes a characteristic *cdiBAI* operon and additional distant *cdiI* variants. We identified internal promoters driving supplementary *cdiI* expression, which may protect bacterial cells from multiple CDI toxins. Further analyses revealed that the CDI locus contributes to biofilm formation. Phylogenetic analyses allowed inference of the likely origin of the CDI module in *Cronobacter* spp. Our results indicate that CDI-like loci are conserved features of pVirCro plasmids across *Cronobacter*, although they are frequently lost in *C. sakazakii*. These findings highlight the evolutionary stability and potential functional significance of CDI systems within this genus.

A063

Beyond Resistance: Toxin-Antitoxin-Mediated Stress Adaptation and Persistence in *Escherichia coli* and *Morganella morganii* isolated from Ghanaian Hospitals

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Abstract

The transient survival of bacterial subpopulations during antibiotic exposure remains a major obstacle to the control of chronic and recurrent infection. Increasing evidence shows that persistence is a transcriptionally regulated state driven by global stress responses and toxin-antitoxin (TA) systems, which promote metabolic slowdown and antibiotic tolerance. However, the molecular basis of persistence in clinical isolates, particularly *Escherichia coli* and the understudied *Morganella morganii*, is poorly understood within African healthcare settings.

This study examined the role of TA systems and stress adaptation in persistence using ciprofloxacin-induced persisters, temperature-tolerant cells, and stationary-phase controls. Bulk RNA sequencing and network analyses revealed distinct transcriptional signatures in persister populations, with over 500 differentially expressed genes (DEGs). Persisters showed strong activation of stress response and TA loci, including *hha/tomB*, *hipB/hipA*, and *higB,* while classical resistance genes such as *gyrA*, *acrA*, and *acrB* showed moderate upregulation. Temperature-adapted cells exhibited broader reprogramming of stress and membrane-stability pathways and upregulated of *parC/parE*. Network mapping highlighted TA interactions with ribosomal and transport-associated hub proteins in *E. coli*, and motility-related proteins in *M. morganii*.

These findings demonstrate that the genetically resistant clinical isolates use TA-mediated stress networks to enhance survival under antibiotic pressure. This dual strategy underscores the complexity of bacterial tolerance and identifies TA systems as potential targets for novel therapeutic interventions against persistent and multidrug-resistant infections.

A064

Genomic epidemiology of African syphilis as a window into global transmission dynamics of Treponema pallidum

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Abstract

Background:

Syphilis rates have increased dramatically worldwide over the past two decades. Genomic surveillance can inform control and intervention strategies, whilst ensuring vaccines have broad utility. Although more than 1,000 *Treponema pallidum* genomes are available from high-income countries, genomic data from Africa remain limited.

Methods:

We combined samples from a genital ulcer aetiology study conducted in Botswana, Ghana, Uganda and Zimbabwe (2022-2024) with samples from national syphilis surveillance in South Africa (2006-2023) to generate 147 novel African *T. pallidum* genomes. Combined with 167 publicly available African genomes and 1062 genomes from 24 non-African countries, we performed contextual population genomic analyses to understand *T. pallidum* genomic diversity and transmission within and between African countries and globally.

Results:

Contrasting with previous studies showing globally homogeneous *T. pallidum*, we found remarkable diversity amongst African *T. pallidum*. We identified 25 sublineages from 6 African countries, compared to 36 sublineages from 24 non-African countries. Sharing of sublineages between African countries and the rest of the world was uncommon, with 83.8% of African syphilis caused by locally circulating sublineages exclusive to Africa. Only 20.1% of African syphilis was resistant to macrolides compared to a global average of 68.6%, and resistance was strongly associated with introduction of global sublineages into Africa.

Conclusion:

African *T. pallidum* is characterised by locally circulating strains not found globally. Since inter-country sublineage sharing within Africa is low, cataloguing African *T. pallidum* diversity will require intense local sampling in many countries. These findings will inform ongoing strategies for genomic surveillance and vaccine design.

A065

Assessing the accuracy of commonly used bioinformatic tools to predict antibiotic resistance phenotypes from Helicobacter pylori genomes.

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Abstract

The majority of stomach cancers are attributed to *Helicobacter pylori* infection, but *H. pylori* eradication is impeded by growing levels of antimicrobial resistance (AMR). Since *H. pylori* is slow-growing and challenging to culture for downstream phenotypic antibiotic susceptibility testing (AST), whole-genome sequencing offers a promising alternative for genotype-based AMR prediction.

This project aimed to evaluate the accuracy of publicly available AMR prediction tools and explore genotype-phenotype associations towards improving predictive power to the main therapeutic antibiotics clarithromycin, metronidazole, tetracycline, levofloxacin, and amoxicillin.

A curated collection of 1,634 *H. pylori* genomes with AST data was gathered, combining our own data with publicly available data identified through systematic literature searches. The genomes were processed through the AMR predictive bioinformatic tools ResFinder, AMRFinderPlus and RGI (Resistance Gene Identifier), and compared to AST data. Across all antibiotics, the best-performing tool, RGI, correctly predicted AMR phenotype in 43.8% of cases, indicating an urgent need for better AMR predictive tools for *H. pylori*.

To improve prediction of AMR phenotypes from *H. pylori* genomes and uncover novel associations of genes conferring resistance, we performed regression analysis using Random Forests on the presence patterns of accessory genes, aiming to identify gene combinations that improve prediction of AMR. Our machine learning approach suggests that leveraging gene combinations rather than single markers could enhance AMR prediction in *H. pylori,* following refinement and validation of gene sets. Ultimately, this work highlights the potential of integrating machine learning with genomic data to overcome current limitations in AMR detection, to improve treatment outcomes.

A066

Genetic changes in metabolic genes aiding the emergence of multidrug resistant *Escherichia coli*

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Abstract

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

Here, we study the differential evolution in MDR *E. coli* by examining the allelic variation within core genes. We use this to evaluate the levels of selection acting on each gene whilst looking for unique patterns of selection.

Focussing on ST131, a pandemic MDR *E. coli* lineage, we compared variation in core genes and observed the distribution of these alleles across the ST131 phylogenetic tree. Comparing the allelic diversity between clades identified key genetic changes in metabolic genes, which could be of importance in the emergence of MDR pathogens. Further investigation into the specific variation in these alleles was carried out to understand the affect these changes may have. The investigation then incorporated further *E. coli* lineages to establish if this pattern of selection was reflected elsewhere in the species.

Overall, we present evidence that changes to metabolic genes may have important ramifications for the success of this MDR lineage.

A067

Starvation and the evolution of sporulation and germination behavior in *Bacillus subtilis*

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Abstract

Endospore-forming bacteria are well adapted to fluctuating environments. However, when nutrients are abundant, the ability to sporulate is quickly evolutionarily reduced or lost. Therefore, we hypothesized that frequent enough nutrient renewal would reduce spore production, and that infrequent enough nutrient renewal would not. We employed batch culture of *Bacillus subtilis* strain 168 for eleven transfers to test the effects of 36h, 56h, and 84h nutrient cycles on the evolution of the behavior of sporulation, germination, and growth. We also investigated the role of cannibalization in the 56h selection using knock-out strains for three separate cannibalizing factors. Nearly all our replicates evolved in parallel within each selection experiment. The 36h and 56 selections reduced sporulation efficiency without loss of sporulation ability. However, the 56h selection uniquely developed dimorphism of colony types across replicates. The dominant morphology had reduced sporulation, and the rarer had accelerated sporulation and highly efficient germination. The 84h selection was infrequent nutrient renewal, resulting in increased spore production. The cannibalizing factor knock-out strains maintained their spore production, suggesting that cannibalism extends the viability of vegetative cells in stationary phase. Genetic changes underlying phenotypes generally occurred independently in the same genes, with a few rarer changes creating similar outcomes. These results suggest that short-term adaptations of *Bacillus subtilis* to cyclical nutrient availability are largely predictable, with a threshold of nutrient availability below which sporulation won't be reduced.

Session : Infection Forum

A068

Speed Versus Sensitivity: Evaluating Direct quantitative Polymerase Chain Reaction for Respiratory Pathogens in Cystic Fibrosis

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Abstract

Background: Rapid and accurate detection of respiratory pathogens is critical for managing pulmonary infections in people with cystic fibrosis (CF). While culture remains the gold standard, quantitative real-time PCR (qPCR) may enable earlier detection of low-burden infections but requires DNA extraction. This study evaluated a quick, extraction-free qPCR method (direct qPCR) against standard real-time qPCR (with DNA extraction) and traditional culture.

Methods: Sputum samples (n=79) were collected from paediatric CF patients at Galway University Hospital (GUH). Each sample was tested using culture, real-time qPCR and direct qPCR. Validated qPCR assays for the detection and quantification of *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Staphylococcus aureus* were used. Detection performance was assessed by calculating sensitivity and specificity and statistical comparisons were performed. Ethical approval was obtained from the GUH Clinical Research Ethics Committee.

Results: Direct qPCR showed variable sensitivity. For *P. aeruginosa*, no positives were detected despite 16 samples confirmed by real-time qPCR (sensitivity = 0%). For *S. aureus*, sensitivity was moderate (56.2%), while *H. influenzae* detection was higher (82.8%). However, negative predictive values were low across all pathogens. McNemar's test revealed significant discrepancies between quick and real-time qPCR ($p < 0.05$), with poor agreement ($\kappa \leq 0.20$). A non-significant correlation was found between real-time qPCR and direct qPCR.

Conclusions: Although rapid, extraction-free qPCR is not suitable for use in this research application due to poor sensitivity. Real-time qPCR with DNA extraction remains essential for accurate detection and clinical decision-making. Optimisation of extraction-free workflows may eventually enable rapid reliable testing in CF care.

Session : Knocking Out AMR

A069

Root exudates of Withania somnifera can prevent the transmission of antibiotic-resistant Enterobacterales in soil and plants

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Abstract

Soil-dwelling enteric pathogens are indicators of sewage or fecal matter contamination. When such organisms enter agricultural fields, they colonize crops, causing pre-harvest contamination of food products. Consumption of such contaminated products can lead to disease outbreaks. These organisms have developed antibiotic resistance due to extensive horizontal gene transfer, making them difficult to treat. Therefore, preventing pre-harvest contamination of food products is essential for public health. The Coochbehar district of India receives >3000mm of rainfall annually, rendering anaerobic conditions in soil, creating an ambient microenvironment for the survival of *Citrobacter* and *Enterobacter sp.,* which are resistant to many antibiotics. While screening for native Enterobacterales in this region, a serendipitous observation was made, wherein the rhizosphere of the *Withania somnifera* plant had a negligible number of enteric bacteria. Artificially inoculated *Citrobacter* sp. was unable to colonize the roots of *Withania*. Water-soluble exudates had prolonged antimicrobial activity *in vitro*, proving that the antimicrobial molecule is secretory in nature. The bactericidal activity was due to the change in the membrane potential specific to bacteria, causing oxidative stress. This was verified using a membrane-potential specific dye uptake. Moreover, the antimicrobial activity spread through the soil via irrigation water to the neighbouring plants. Thus, it was concluded that growing *Withania* alongside susceptible plants like *Vigna radiata* can prevent the colonization of the rhizosphere, minimizing the risk of pre-harvest contamination of fresh produce. To the best of our knowledge, this is the first report of using *Withania* as a biocontrol plant against Enterobacterales.

A070

Phenolic-rich blueberry extract enhances gentamicin efficacy against multidrug-resistant Acinetobacter baumannii BAA1605 via efflux inhibition and suppression of antibiotic-induced metabolic activation

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Abstract

Antibiotics are effective in treating bacterial infections, but their widespread use has spurred antibiotic resistance that is linked closely with human disease. While dietary components are known to influence the gut microbiome, specific effects on the gut resistome—the collection of antibiotic-resistant genes in the gut—remain underexplored. This review outlines the mechanisms of antibiotic action and the development of resistance, emphasizing the connection between the gut resistome and human diseases such as metabolic disorders, cardiovascular disease, liver disease, and nervous system disorders. It also discusses the effects of diet habits and dietary components, including bioactive macronutrients, phytochemicals, and probiotics, on the composition of the gut resistome by enhancing antibiotic efficacy and potentially reducing resistance. This review highlights the emerging trend of increasing interest in functional foods aimed at targeting the gut resisome and a growing focus on bioactive plant compounds with potential to modulate antibiotic resistance.

A071

Mapping the Dissemination of Multidrug-Resistant Staphylococcaceae in Veterinary Healthcare

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Abstract

Veterinary hospitals are hidden sites where multidrug-resistant (MDR) Staphylococcaceae can persist and spread, contributing to hospital-acquired infections and creating silent dissemination pathways that extend far beyond animal care, representing a One Health threat. This study aimed to map the dissemination of MDR Staphylococcaceae in a relevant Brazilian veterinary teaching hospital. A total of 71 swab samples were collected from 15 sites (wash bottle, computer, door handles, light switches, surgical table, anesthesia machine, sink, surgical light, doors, phone, patient table, drawers, faucet, cage, and support table) across 12 examination rooms and two surgical rooms. Samples were cultured on mannitol salt agar, and colonies were identified by MALDI-TOF. Antimicrobial susceptibility was evaluated by disk-diffusion. Fifty-seven isolates belonging to 22 species were obtained. Staphylococcaceae accounted for 29.8% (17/57), including six species and one *Staphylococcus* spp. *S. pseudintermedius* was the most prevalent species (58.8%), followed by *S. simulans* (11.8%), and *S. haemolyticus, S. epidermidis, S. hominis*, and *Mammalicoccus sciuri* (5.9% each). *S. pseudintermedius* was also the most widespread, particularly on computers (50%), door handles and patient tables (20% each), and light switches (10%), being detected in eight rooms (66.7%). Most isolates were resistant to penicillin (76.5%), erythromycin (64.7%), tetracycline, clindamycin, and enrofloxacin (47% each). Overall, 53% of isolates were MDR, mainly *S. pseudintermedius* (70% of the isolates). These findings demonstrate that veterinary teaching hospitals can act as reservoirs for MDR Staphylococcaceae, emphasizing their role as potential sources of hospital-acquired infections and One Health dissemination pathways that require urgent surveillance and control measures.

A072

The enemy at the gates? To what extent do wildlife carry shared antimicrobial resistance?

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Abstract

Antimicrobial Resistance (AMR) is a key threat to global health, with the World Health Organisation classifying third generation cephalosporin resistant *E. coli* as a critical priority pathogen. It is becoming increasingly apparent that a holistic, One Health approach is needed to understand the dynamics of how AMR is disseminated through the groups that represent the aspects of One Health. The WHO created the standardised 'Tricycle protocol' as a surveillance tool to observe changes in abundance and distribution of AMR within clinical, wastewater and poultry sources.

This research uses the Tricycle protocol as a foundation for a longitudinal study that aims to determine the abundance, distribution and dissemination of ESBL *E. coli* (ESBL-Ec) both temporally and spatially from different sources representative of One Health: wildlife and environment, human and livestock. Two poultry farms were selected based on location near a sewage treatment works or isolated from human influencer sites. Wildlife, water and environmental samples were collected quarterly over a year. Poultry samples were collected continually, representing the start, middle and end of life.

Of the 1328 isolates recovered over the year, 46 grew in the presence of cefotaxime during initial isolation, with the majority (44/46) recovered from the water and wildlife found at the site near the STW. No ESBL-Ec was recovered from poultry samples.

The lack of ESBL-Ec recovered from poultry, rodents and invertebrates suggests that there are other more significant sources of ESBL-Ec such as rivers receiving STW effluent and mammals within the vicinity of these poultry units.

A073

Integrating (meta)genomic data into semi-quantitative risk assessment of antimicrobial resistance in the broiler meat processing chain

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Abstract

Antimicrobial resistance (AMR) remains a major global threat to food safety and public health. Next-generation sequencing (NGS) technologies, including whole-genome sequencing (WGS) and shotgun metagenomics, enable high-resolution detection and characterisation of antimicrobial resistance genes (ARGs) in both isolates and complex microbial communities. In this study, broiler caecal and neck skin samples from Slovenian and Spanish slaughterhouses were analysed using culture-based methods, metagenomic sequencing (Illumina), and WGS of selected zoonotic isolates (Oxford Nanopore and Illumina). A modular, semi-quantitative AMR risk assessment model was developed, integrating genomic data with key risk determinants.

The model successfully differentiated AMR risks across processing stages and between countries, highlighting the influence of production practices on resistome composition. Despite challenges such as limited quantitative exposure data and high host DNA background in neck skin samples, integrating (meta)genomic evidence and epidemiological data provides a transparent and flexible approach to AMR risk ranking. This framework supports evidence-based decision-making and targeted mitigation strategies throughout the food chain, advancing One Health-aligned surveillance and risk management.

A074

Multidrug resistance in poultry meat: a comparison between conventional and antibiotic-free production systems

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Abstract

Antimicrobial resistance (AMR) represents a critical global public health threat, with increasing concern about its emergence within animal production. The routine use of antibiotics in conventional poultry farming exerts selective pressure that favors multidrug-resistant (MDR) bacteria. Antibiotic-free (ABF) production systems have been proposed as a promising alternative to mitigate this issue. This study aimed to compare the occurrence of MDR *Salmonella* spp., *Escherichia coli*, and *Enterococcus* spp. in broilers raised under conventional and ABF systems at different production stages. Samples were collected at four key points: before housing (AA), mid-housing (MA), end of housing (FA), and slaughter (AB). The bacterial were isolated, identified, and tested for antimicrobial susceptibility using standardized methods. MDR was defined as resistance to three or more antimicrobial classes. The frequency of MDR isolates was compared between production systems using appropriate statistical tests. Overall, MDR rates were higher in the conventional system. Among *Salmonella* spp., 99.45% of isolates from conventional broilers were MDR, compared with 50% in ABF broilers ($p = 0.0214$). For *E. coli*, 95.2% of conventional isolates and 70.63% of ABF isolates were MDR ($p < 0.0001$). *Enterococcus* spp. exhibited high MDR rates in both systems, with no significant difference ($p > 0.9999$). These results indicate that ABF production significantly reduces MDR in *Salmonella* spp. and *E. coli*, particularly in earlier production stages, while *Enterococcus* spp. persistence may reflect intrinsic or environmental factors. ABF systems thus represent an effective strategy to mitigate AMR in poultry production and safeguard public health. Funding FAPESP (Processes 22/03062-6, 23/01185-6, 23/01195-1).

A076

The Omp85 family protein, TamA, exhibits characteristics of a suitable drug target against Pseudomonas aeruginosa

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Abstract

The outer membrane (OM) of Gram-negative bacteria is crucial for cell stability and virulence and acts as a permeability barrier. The biogenesis, assembly, and regulation of proteins in the OM are therefore attractive areas of study that could lead to identifying novel drug targets. The Translocation and Assembly Module (TAM), composed of TamA and TamB, facilitates the insertion of some β-barrel proteins into the OM of *Escherichia coli* and *Klebsiella pneumoniae*, and has also been implicated in lipid homeostasis. However, its role in *Pseudomonas aeruginosa* remains mostly uncharacterized. To investigate the TAM's function and drug target potential in *P. aeruginosa*, we generated both single- and double-gene TAM knockouts and assessed their fitness using competition growth assays against wild-type (WT) strains. The WT significantly outcompeted the TAM mutants, indicating a fitness defect. Proteomic analysis revealed surprisingly similar profiles between WT and the double knockout strains, while single knockouts showed changes in OM proteins and reduced expression of flagellar components consistent with attenuated swimming motility observed in ΔtamA. Single mutants exhibited differential levels of expression of lipoproteins of the β-barrel assembly machinery suggesting compensatory OM remodelling. In vivo infection assays using *Galleria mellonella* larvae demonstrated significantly higher survival rates when infected with TAM mutants, with *tamA* mutants showing the greatest attenuation in virulence. Our findings demonstrate a role the TAM plays in *P. aeruginosa* virulence and identify TamA as a potential drug target for the development of new antimicrobial therapies.

A077

Antimicrobial peptides as a Promising Antibiotic Alternative to Combat Periodontal Disease

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Abstract

The indiscriminate use of antibiotics has led to a global increase in bacterial resistance, posing a significant challenge in treating infections, particularly in the dental field. This has prompted a search for alternative antimicrobial agents. Antimicrobial peptides (AMPs) are a promising solution because they work by disrupting bacterial membranes, a mechanism that makes the development of resistance less likely.

This study aimed to investigate two synthetic derivatives of the human antimicrobial peptide LL37, FK13a1 and KR12-NH2. The primary goal was to determine their ability to inhibit the growth of key periodontal pathogens, including F. nucleatum, P. gingivalis, and S. oralis. Crucially, the research also examined their efficacy in the presence of cigarette smoke extract to assess their potential for use in smoking patients.

The findings revealed that both peptides were effective against planktonic bacteria, with FK13a1 demonstrating superior performance. A notable discovery was that cigarette smoke extract itself significantly reduced bacterial growth. When combined, the peptides and cigarette smoke extract showed an enhanced inhibitory effect. When tested on single-species biofilms, both FK13a1 and KR12-NH2 successfully reduced both biomass and thickness. However, their effectiveness was less consistent against more complex multi-species biofilms, where they were only able to reduce biomass without a significant impact on thickness.

In conclusion, the findings suggest that FK13a1 and KR12-NH2 are effective at inhibiting periodontal pathogens and remain active in a smoking environment. While their effectiveness against complex biofilms was not absolute, these results position them as a promising alternative to conventional antibiotics.

A078

Precision in flow: rapid microfluidics for uropathogen detection

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Abstract

With bacterial evolution outpacing the development of new treatments, antimicrobial resistance (AMR) continues to redefine the management of common infections. Urinary tract infections (UTIs), once readily treatable, are increasingly caused by resistant uropathogens that limit effective therapeutic options. The gold standard for UTI diagnostics relies on bacterial incubation and growth, leaving up to a week before pathogen presence is confirmed. This delay often leads to empirical antibiotic prescribing and missed opportunities for targeted care. Our work aims to replace these time-intensive methods with a rapid, reliable approach for early infection detection to support timely clinical decision-making and responsible antibiotic use.

More specifically, a label-free microfluidic technology was proposed that harnesses inertial forces to focus particles at precise locations and hence, enrich pathogens from urine rapidly, at high throughput. To assess its performance, model polyacrylamide hydrogels ranging from 1.72 to 328.7 μm^2 were used, reflecting the size distribution of UTI-positive samples, with an average area of 3.91 μm^2, closely matching the dimensions of uropathogenic bacteria. Upon design and flow optimisation, we demonstrate that hydrogels can be tightly focused in the microfluidic channel, allowing a 10X enrichment rate in one cycle. Using these optimised parameters, uropathogenic *E. coli* (heat-killed and alive) successfully showed a 263.6% increased bacterial content, compared with unprocessed samples. Successful viability testing post-enrichment was also confirmed, offering space for antibiotic sensitivity testing.

These critical preliminary results highlight culture-independent enrichment as a key step toward rapid diagnostics, facilitating targeted treatment, improving patient outcomes, and reducing the global burden of AMR.

A079

Tracking AMR in a Changing Climate: Plasmid-Mediated Antibiotic Resistance in Flooded Agricultural Soils in Ireland

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Abstract

Soils represent complex and diverse microbial habitats and serve as important environmental reservoirs for antimicrobial resistance genes (ARGs). Environmental disturbances such as flooding can both influence microbial community structure and the potential for horizontal gene transfer (HGT), thereby affecting the distribution and mobility of ARGs. This study investigated the presence and plasmid-mediated dissemination of antibiotic resistance in agricultural soils under both flooded and non-flooded conditions across agricultural sites in Ireland.

Quantitative PCR (qPCR) revealed a diverse array of clinically relevant ARGs, including genes conferring resistance to fluoroquinolones (*qepA*), aminoglycosides (*aadA7*), β-lactams (*blaSFO*), vancomycin (*vanA*), macrolides (*erm(31*)), and carbapenems (*cphA1*). Mobile genetic elements such as *Tn5403* and *tnpA* were also detected, indicating the potential for gene mobility. Exogenous plasmid capture experiments confirmed the transferability of resistance plasmids via conjugation, with ampicillin- and tetracycline-resistant transconjugants recovered from both flooded and non-flooded soils. Plasmids were subsequently extracted and sequenced using Oxford Nanopore Technology, giving a deeper insight into the plasmid diversity and potential mobility of ARGs within and between the soil conditions. Results indicate site-specific variation in resistance gene abundance and plasmid activity, underlining the influence of environmental conditions on ARG prevalence and dissemination.

This work highlights agricultural soils as dynamic reservoirs of mobile resistance and the importance of environmental AMR surveillance, particularly in the context of climate-driven events such as flooding.

A080

Biofilm formation, antimicrobial susceptibility and virulence factors of uropathogenic *Escherichia coli* isolated from renal patients.

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Abstract

Advanced virulence factors in UPEC strains facilitate their ability to enter and colonize the host, interfere with the host's defense system, damage human tissue, and incite an unpleasant inflammatory response in the host. The purpose of this study was to identify biofilm formation, antimicrobial susceptibility and related virulence factors of uropathogenic *Escherichia coli* isolated from renal patients. A total 590 mid-stream urine sample were processed. 176 UPEC isolates were identified and subjected to further laboratory analysis. The prevalence of uropathogenic *E. coli* was found to be 73.94% among which 78.40% isolates were multi-drug resistant. Polymyxins were mostly effective class of antibiotics with sensitivity of 93.18% for both Polymyxin B and Colistin followed by Meropenem (72.73%) and Amikacin (73.86%). The majority of the isolates showed highest resistance towards Cefixime (82.95%), Ceftazidime (76.14%) and Ciprofloxacin (71.59%) respectively. 80.68% of isolates were invitro biofilm producers. Multi-drug resistance was more common among the biofilm producers with total of 77.46%. Biofilm producers showed maximum resistance towards Cefixime (88.50%) followed by Ceftazidime (80.28%).Out of total isolates, 62.5% produced alpha hemolysis; 61.81% were positive for mannose resistant haemagglutination and 38.18% were positive for mannose sensitive haemagglutination. Biofilm formation associated with mannose resistant haemagglutination was 76.47% while 85.71% of mannose sensitive haemagglutination was associated with biofilm producers. The study shows that the ability to produce biofilm and express virulence factors varies among the strains of uropathogenic *E. coli* and multidrug resistance is more prevalent among those positive for expression of virulence factors.

A081

Host-pathogen interactions shape the evolution of antimicrobial resistance

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Abstract

Antimicrobial resistance (AMR) poses major therapeutic challenges, particularly in chronic biofilm-associated infections. Using a murine *Pseudomonas aeruginosa* biofilm lung infection model, we previously demonstrated rapid ciprofloxacin (CIP) resistance emergence after two administrations in one day, primarily via efflux pump regulator mutations. Here, we investigated resistance evolution under extended CIP exposure (four administrations over two days post-infection). Increased dosing accelerated resistance development, resulting in elevated minimum inhibitory concentrations (MICs). Resistance evolved through an initial mutation-driven tolerance phase, followed by full resistance associated with concurrent mutations in *nfxB* (efflux pump regulator) and *gyrA* (CIP target), consistent with clinical CIP-resistant isolates. Notably, high CIP MICs correlated with collateral sensitivity to tobramycin and aztreonam, revealing a trade-off in resistance pathways. Host response analysis showed elevated cytokine levels in early passages compared to later ones, and histopathology revealed strong neutrophilic inflammation and goblet cell development in untreated mice, resembling clinical biofilm lung pathology. CIP treatment attenuated inflammatory responses. Collectively, this optimized *in vivo* biofilm lung infection model captures both AMR evolution and host-pathogen dynamics, offering a valuable platform for translational research and the development of novel therapeutic strategies against biofilm-associated lung infections.

A082

Linking socioeconomic deprivation to antimicrobial resistance exposure risks in the aquatic environment.

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Abstract

When sewage, industrial waste and agricultural effluent enter the aquatic environment, the resulting cocktail of antimicrobial compounds, pathogenic micro-organisms and antimicrobial resistance (AMR) genes disrupts ecological processes and drives the development of drug-resistant microbes. A rich tapestry of scholarship and activism demonstrates that marginalised communities around the world are disproportionately exposed to environmental hazards, including water pollution. To date, however, the effects of these environmental inequalities on marginalised communities' risk of exposure to AMR in aquatic systems have been critically overlooked. To address this knowledge gap, we conducted a systematic synthesis of published wastewater sampling data to build a dataset describing the prevalence of AMR in aquatic environments across the UK. We are in the process of combining this dataset with geospatial information on socioeconomic deprivation using ArcGIS to map AMR sampling site data onto Index of Multiple Deprivation deciles. This will enable us to assess whether more deprived areas of the UK experience a greater burden of environmental AMR; it also facilitates a critical examination of why environmental inequalities have been largely neglected in AMR research. Our findings will illuminate differential exposure risks posed by AMR in aquatic systems and lay the groundwork for future work on the interlinked geographical and sociodemographic inequalities shaping its impact.

A083

Surveillance of sanitary ware in 3 Irish hospitals for carbapenem resistant Enterobacterales and analysis of their resistance profiles and genomes

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Abstract

Sanitary ware in three geographically distinct hospitals in the Republic of Ireland was screened for carbapenemase producing Enterobacterales (CPE) over three months with one-off sampling six and ten months after initial sampling. Swab samples from the sink drain, shower drain and toilet, and wastewater from the shower drain were taken by hospital staff and delivered to the university for processing. Culture-based methods were utilised to isolate presumed CPE using EMB agar and Simmons citrate agar with 1% myo-inositol impregnated with imipenem according to the EUCAST guidelines to isolate presumed carbapenem resistant *Escherichia coli* and *Klebsiella* spp. respectively. The species were identified using 16S rRNA sequencing. 933 isolates were obtained of which 171 were confirmed to be Enterobacterales. Disc diffusion assays were performed on the confirmed Enterobacterales against ampicillin, cefotaxime, ceftazidime, imipenem, meropenem, ciprofloxacin, amikacin and kanamycin. 96% of isolates were resistant to ampicillin, 76% were resistant to cefotaxime, 77% were resistant to ceftazidime, 62% were resistant to imipenem, 30% were resistant to meropenem, 29% were resistant to ciprofloxacin, 2% were resistant to amikacin and 12% were resistant to kanamycin. Whole genome sequencing was also performed. This shows the limitations of culture-based methods as most of the isolates were not Enterobacterales.

A084

Shotgun Metagenomic Analysis of Zoonotic Pathogens and Antimicrobial Resistance Profiles in White-tailed Deer (Odocoileus virginianus) Feces

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Abstract

The natural environment is increasingly recognized as a critical sector for the evolution and spread of antimicrobial resistance (AMR) drivers and pathogen emergence. Yet, the role of wildlife in these processes remains underexplored. Although the epidemiological significance of white-tailed deer (WTD) in vector-borne zoonoses has been well established, the bacterial pathogens and resistance profile directly associated with these animals are scarcely reported. This study used shotgun metagenomics to characterize microbial composition, pathogen presence, and AMR profiles in the fecal samples of WTD as a wildlife model.

33 zoonotic pathogens were detected, including all ESKAPE pathogens and several foodborne pathogens such as *Bacillus cereus*, *Salmonella* spp., *Campylobacter* spp., *Clostridioides difficile*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, enteropathogenic *Yersinia* spp., and *Listeria monocytogenes*. 611 high-quality antimicrobial resistance genes (ARGs) conferring resistance to numerous medically important antimicrobial classes were identified, with macrolide, aminoglycoside, and multidrug resistance genes being the most prevalent. Metagenome-assembled genome (MAG) analysis confirmed the presence of pathogenic species and ARGs, including a multidrug-resistant *Escherichia coli*MAG carrying 37 ARGs and 106 virulence factors. *Listeria* and *E. coli* isolates recovered from WTD fecal samples were whole-genome sequenced to obtain in-depth genome-resolved insights. Two *Listeria welshimeri* strains were identified, harboring three clinically relevant ARGs and 15 virulence factors associated with predicted human pathogenicity.

Together, these findings provide valuable insights into the underexplored relevance of WTD in the evolution and spread of AMR and zoonotic pathogens, highlighting the potential of WTD to serve as a sentinel species for integrated One-Health surveillance at the wildlife-environment-human interface.

A085

Identification of novel drug resistance mechanisms in *Acinetobacter baumannii* using TraDISort approach

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Abstract

The rapid rise of multidrug-resistant pathogens poses a critical global health threat, with *Acinetobacter baumannii* emerging as one of the leading bacterial pathogens causing hospital-acquired infections. Understanding the genetic basis of antibiotic tolerance is essential for developing new therapeutic strategies. In this study, we used TraDISort, a high-throughput method that combines transposon-directed insertion site sequencing (TraDIS) with fluorescence-activated cell sorting (FACS), to identify genes involved in resistance to the antimicrobial dyes acriflavine and rhodamine 6G. Acriflavine, an acridine derivative, and rhodamine 6G, a xanthene dye, both impair bacterial viability by intercalating into DNA and disrupting essential cellular processes. Rhodamine 6G is also a known substrate for multiple drug efflux pumps, and we aimed to explore whether novel efflux pump families exist in *A. baumannii*. A dense transposon mutant library of *A. baumannii* ATCC 17978 was exposed to sub-inhibitory dye concentrations and sorted based on fluorescence intensity to separate bright and dim populations. Sequencing and comparative insertion analysis identified genes linked to efflux, outer membrane integrity, and polysaccharide biosynthesis. Mutants with disruptions in efflux or envelope-associated genes showed distinct fluorescence shifts, reflecting changes in membrane permeability and transport. Additionally, several novel hypothetical genes were identified, suggesting new directions for understanding resistance mechanisms. Overall, this study demonstrates the power of TraDISort to uncover both known and novel determinants of antimicrobial resistance, providing a functional genomics framework for mediating antimicrobial resistance challenges in *A. baumannii*.

A086

Megaplasmids and Microbial Survival: Elucidating Preservative Tolerance in *Burkholderia cepacia* complex species

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Abstract

Bacteria belonging to the genus *Burkholderia* were the third most common bacterial contaminants found in home and personal care products over the past decade. To mitigate contamination, preservatives, such as sodium benzoate and multifunctional ingredients, such as EDTA are incorporated into these products. However, *Burkholderia* species have demonstrated a notable tolerance to these agents.

Previous studies have highlighted the third replicon of *B. cepacia* complex species as a key contributor to virulence and pathogenicity in both clinical and environmental isolates. We hypothesise that this replicon may also confer resistance to preservatives and enable fitness in industrial settings. In this work, we investigated how the presence of the third replicon may confer or enhance tolerance to EDTA, sodium benzoate, and their combination.

To address this, we created third replicon mutants in a range *B. cepacia* complex species and employed checkerboard assays to assess the impact of preservatives and multifunctional ingredients on the wild type versus replicon deficient derivatives. An altered phenotype of third replicon mutants in relation to the tolerance of sodium benzoate was identified. The genetic basis of this is being examined using whole genome sequencing to identify candidate genes located on the third replicon, and will be followed up through transcriptomic analysis of gene expression (RNA sequencing). Understanding how *B. cepacia* complex species tolerate preservative systems will inform the selection of more effective and environmentally sustainable formulations for home and personal care products.

A087

Regulation of Bacterial Biofilm Dispersal by Multi-Domain Red-Ox Sensing Proteins

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Abstract

Pseudomonas aeruginosa is a gram-negative bacterium ubiquitously present in the environment. It is an opportunistic pathogen and is part of the ESKAPE group, which are the primary sources of nosocomial infections in healthcare environments. *P. aeruginosa* is shown to have a pervasive expression of antibiotic resistance mechanisms and demonstrates a robust capability for biofilm formation. As such, the presence of *P. aeruginosa* has been correlated with a poorer prognosis in with immunocompromised and cystic fibrosis patients.

Biofilms are a key mechanism in antibiotic resistance. Within biofilms there are redox gradients, occurring due to factors such as oxygen and nutrient availability. Redox activity plays a key role in biofilm dispersal. Examples include production of reactive oxygen species by neutrophils which mediate destruction of biofilm structures or nitric oxide which induces biofilm dispersal.

In this project we focus on the study of several key membrane proteins which sense nitric oxide and redox stress, with a role in regulating production of Cyclic diguanylate monophosphate (C-di-GMP), a signaling molecule which upregulates biofilm formation.

In our studies we will be carrying out time resolved confocal microscopy of continuous flow cell cultures in conjunction with infection models, protein biochemistry, structural bioinformatics and crystallography would facilitate more specific targeting of redox sensors. In this combined approach we can characterise potential target proteins and their effects on biofilm formation.

Elucidating these target regions would facilitate the disruption of key biofilm signaling pathways and localised pools of C-di-GMP production which would improve efficacy of antibiotics and immune clearance.

A088

Can we mitigate antimicrobial resistance in pig slurry for sustainable agriculture?

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Abstract

Animal slurry is an economical and sustainable fertiliser that can reduce the agricultural carbon footprint. However, its use has been associated with the dissemination of antimicrobial resistant bacteria and genes, posing a significant One Health risk. *Escherichia coli* and plasmid-mediated antimicrobial resistance genes (ARGs) are of particular concern due to their potential transfer between animals, humans, and the environment. Previous work from our laboratory using metagenomics and high-throughput qPCR demonstrated that extended storage and composting effectively reduce ARGs and mobile genetic elements in pig slurry, but these approaches cannot resolve strain-level dynamics.

In this study, I characterised culturable *E. coli* isolated from these treatments to better understand resistance dynamics. Antimicrobial susceptibility testing was performed on isolates from stored (n = 211) and composted (n = 61) manure. Whole-genome sequencing (Illumina) on all isolates determined sequence types, serotypes, ARGs and plasmid replicon types. Plasmid sequences from WGS were separated from chromosomal sequences and analysed by MASH pairwise distance analysis to identify unique plasmids. Selected plasmids underwent Oxford Nanopore Sequencing followed by hybrid assembly.

Diverse *E. coli* populations were detected, with common co-resistance to ampicillin, tetracycline, sulphonamide, and trimethoprim, largely encoded on plasmids. Over time, both composting and storage treatments led to a significant reduction in ARG abundance, correlating with a decline in plasmid prevalence.

These findings demonstrate that slurry pre-treatment not only reduces pathogen load but can also diminish plasmid-associated resistance reservoirs, highlighting its potential as a practical intervention to help “knock out” antimicrobial resistance at the agricultural source.

A089

Pilot study of the VIPVis app: Is visual simulation the way forward for effective infection prevention and control training for veterinary staff to improve antimicrobial stewardship?

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Abstract

Infection prevention and control (IPC) is important to reduce the frequency of healthcare-associated infections and to ensure effective care through avoiding treatment complications and combatting antimicrobial resistance. There are few educational tools focusing on IPC which concentrate on antimicrobial stewardship. To address this, we developed Veterinary Infection Prevention through Visualisation (VIPVis); a simulation app utilising engaging graphics, highlighting contextual interactions between humans, animals, and microorganisms within the Veterinary Practice setting to demonstrate where pathogenic spread can occur. We assessed whether using VIPVis improves awareness of IPC and contributes to better IPC practices. Staff were asked to complete three questionnaires: before exposure to VIPVis (Q1), immediately afterwards (Q2), and three months later (Q3). Questionnaires were analysed descriptively due to low participant number; thirteen staff completed Q1, six completed Q2, and four completed Q3. Following the use of VIPVis, risk perception for pathogen spread increased in all 8 assessed behaviours, whilst the frequency of undertaking positive IPC behaviours improved for 7/8 behaviours; cleaning hands after removing gloves decreased. The primary influence reported for undertaking IPC measures was to stop the spread of pathogens, while staff stated that a busy schedule/limited time availability prevented them undertaking IPC measures. Fluctuations in Likert scale scores indicate over-confidence conveyed at the start of the study, which decreased following the use of VIPVis, but increased again at the three-month follow-up. This pilot study has shown potential for VIPVis to contribute to improved antimicrobial stewardship through increased implementation of IPC behaviours in the veterinary practice environment.

A090

AMR Characterisation of Pasteurella multocida isolated from UK livestock (Cattle, Sheep, and Pigs) from 2020–2024.

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Abstract

Pasteurella multocida is a commensal and opportunistic pathogen with a capacity to infect a wide range of hosts across diverse geographical regions, producing varied clinical manifestations. In the UK livestock industry, it is a major cause of respiratory disease in pigs, sheep, and cattle, leading to considerable morbidity, mortality, and economic losses. Despite its significance, systematic monitoring of antimicrobial resistance (AMR) of veterinary pathogens in animals has not been the subject of UK legislation, contrasting with zoonotic bacterial organisms. Currently, AMR monitoring in veterinary pathogens is performed through voluntary scanning surveillance, where only phenotypic methods are used, limiting understanding of its evolutionary dynamics and resistance mechanisms.

This study extends the use of whole-genome sequencing (WGS) and in-silico AMR identification tools to 465 *P. multocida* isolates obtained from clinical samples of cattle, sheep, and pigs across UK farms from 2020 to 2024. Genotypic and phenotypic AMR data are integrated through genome-wide association studies (GWAS) to identify mutations and resistance genes associated with antimicrobial phenotypes. The investigation reveals both horizontally acquired resistance determinants and chromosomal mutations linked to reduced antimicrobial susceptibility. These findings will advance understanding of the genetic mechanisms underpinning AMR in *P. multocida*, strengthen the foundation for WGS-based surveillance as a complement to traditional phenotypic methods, which will contribute to improved precision diagnostics and future epidemiological monitoring in livestock.

A091

Can we detect it? A systematic comparison of strategies for predicting antimicrobial resistance from nanopore sequence data

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Abstract

Background

Faced with a growing antimicrobial resistance (AMR) crisis, there is a pressing need to ensure that the right antimicrobials are used to target the right microbes, at the right time. Clinically, the appropriate antimicrobial is typically selected after samples have been cultured and assessed with antimicrobial sensitivity testing (AST). Nanopore-based metagenomic whole-genome sequencing is a rapid alternative to culture-based diagnosis, using all DNA from patient samples to identify the pathogens present and predict AMR. Thus far, one limitation of this approach has been the inconsistency of *in silico* predicted AMR phenotypes, demonstrated by various recent studies with short read data.

Methods

We used nanopore long reads paired with AST phenotyping results for 201 bacterial samples to benchmark the current performance of *in silico* AMR prediction strategies. We examined the impact of basecalling accuracy, data volume, and genome assembly strategy, as well as comparing the overall performance of eight AMR prediction tools and a variety of AMR databases.

Results

We found that base calling accuracy mode does not affect the accuracy of AMR detection, but genome assembly strategy and data volume both do. Further, prediction tools based around the ResFinder database outperform other tools, albeit with a maximum balanced accuracy of only 0.8 ± 0.02. Some classes of resistance, such as fluoroquinolone, were poorly detected by all tools.

Conclusions

We have performed extensive benchmarking to establish the current best-performing nanopore AMR prediction strategy, but demonstrate that further work is still required to optimise AMR prediction from long read sequence data.

A092

WHAT'S IN THE WATER? A MIXED METHODS EXPLORATION OF THE ONE HEALTH RISKS OF ANTIMICROBIAL RESISTANCE IN SCOTTISH WILD SWIMMING SITES.

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Abstract

Antimicrobial resistance (AMR) poses a critical threat to global health, requiring a coordinated, One Health approach. Although AMR is a natural evolutionary process, its evolution and spread is known to have been accelerated by anthropogenic activities including intensive agriculture and wastewater discharge into the environment. However, the role of the natural environment in AMR proliferation, dissemination, and transmission, along with the corresponding risks to users and the broader environment, has remained underprioritized. Focusing on wild swimming, an activity which occurs at the intersection of One Health spheres, this project aims to investigate the presence of resistant bacteria and antimicrobial resistance genes (ARGs) in inland, freshwater sites in Scotland. This environmental surveillance pilot study will use culture-based approaches in tandem with shotgun metagenomics, and short and long read sequencing. This combination of methods will provide a comprehensive overview of the relative abundance of key bacteria, facilitating the source tracing of resistant pathogens and ARGs of interest. Ultimately, this study is the first step in demonstrating how a holistic One Health approach can more effectively address AMR priorities, leveraging strengthened surveillance, effective communication, and informed policy.

A093

Investigating Antimicrobial Peptides that Affect the Outer Membrane Integrity of Gram-negative Bacteria

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Abstract

The β-barrel assembly machinery (Bam) complex resides within the outer membrane (OM) of Gram-negative bacteria, where it facilitates the folding and insertion of OM proteins. As an essential component for cell survival, the Bam complex presents a promising but underexplored target for antibiotic development. JB-95 is a synthetic β-hairpin macrocyclic peptide proposed to interact with the Bam complex; however, the mechanism of action and potential pathways to resistance remain unclear. To investigate this, we determined the minimum inhibitory concentration (MIC) of JB-95 against *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, and observed antimicrobial activity against all bacteria tested. Further analysis included monitoring the growth of wild-type *E. coli* strains and mutants lacking the components of the Bam complex or OM protein chaperones in the presence of increasing JB-95 concentrations. Mutants lacking protein chaperones SurA and DegP exhibited heightened sensitivity to JB-95 whereas deletion of the Bam accessory lipoprotein BamC slightly increased fitness under JB-95 stress compared to the wild-type strain. To assess resistance development, we used adaptive laboratory evolution by serially passaging E*. coli* and *A. baumannii* in the presence of JB-95. Whole-genome sequencing revealed mutations associated with increased biofilm formation, the DNA-binding protein, H-NS, and the OM phospholipase, PldA. Interestingly, no mutations were observed in Bam complex genes. These findings suggest JB-95 is effective against clinically relevant Gram-negative pathogens, but the precise mechanism of action is still unknown. The absence of Bam-related resistance mutations highlights the need for further investigation into the molecular targets and resistance mechanisms to JB-95.

A094

Baldexin: a potent and synergistic antibiofilm formulation developed from a medieval remedy

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Abstract

A decade ago, we and our collaborators reconstructed a medieval remedy called Bald's eyesalve, and showed that it had strong antibacterial and antibiofilm efficacy, which relied on the presence of multiple chemically-complex natural ingredients. We have now used this recipe as a library of natural products to identify a defined formulation of five compounds that, together, recapitulates and extends the activity of the natural remedy. In this joint presentation, we will present data showing the mechanism of action of our reconstructed Bald's eyesalve on Gram-positive and Gram-negative bacteria. We will then show how we used this data and a rational design approach to create our synthetic formulation, baldexin (Biofilm-Active Low-Dose complEX of Natural products).

Baldexin was shown to have potent biofilm eradication activity against both methicillin-susceptible and -resistant *Staphylococcus aureus*, Acinetobacter baumannii, and *Pseudomonas aeruginosa*, causing at least 4−log10 killing of bacteria in both chronic wound and cystic fibrosis lung biofilm models. This activity requires the presence of all compounds.

We used phenotypic assays and transcriptomics to show that both Bald's eyesalve and baldexin disrupt bacterial membrane potential, leading to irreversible plasma membrane damage in both Gram-positive and negative pathogens. Baldexin also affects nucleotide biosynthesis, pathogenesis, biofilm formation, and efflux. Lastly, we performed artificial selection experiments and found that *S. aureus* and *P. aeruginosa* cannot easily evolve resistance to baldexin.

We aim to develop baldexin into a treatment for chronic bacterial biofilm infections, initially focussing on the creation of an advanced wound care product.

A095

When Life Gives You Sea Lemons: Unlocking the Bioactive Potential of an Antarctic Sea Lemon Microbial Isolate

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Abstract

Antimicrobial resistance (AMR) poses a major global health threat, with recent analyses forecasting up to 39 million cumulative deaths by 2050. Marine-derived microbes represent an underexplored source of bioactive natural products with antibacterial potential. This study investigates the antibacterial activity of the Antarctic isolate *Streptomyces violarus* (identified by 16S rRNA sequencing), strain MM22, recovered from the sea lemon *Marseniopsis mollis*, and examines how culture conditions influence metabolite production.

An OSMAC (One Strain Many Compounds) approach was used to screen MM22 across multiple solid and liquid media against Gram-positive pathogens including *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and *Enterococcus faecalis*. Phenotypic screening revealed pronounced medium-dependent inhibition, with marine agar and International Streptomyces Project Medium 2 (ISP2) supporting the strongest activity. In liquid culture, antibacterial activity was sustained in ISP2 and enhanced in Mueller–Hinton broth (MHB) supplemented with artificial seawater, which restored activity lost in unsupplemented MHB media by day 14.

Crude extracts from ISP2 cultures were fractionated via C18 reversed-phased chromatography, with reproducible activity localised to the 50–75% (v/v) methanol fractions, which is consistent with moderately polar metabolites. These active fractions are currently undergoing mass spectrometric analysis to guide compound identification.

Together, these findings identify MM22 as a promising source of antibacterial metabolites and demonstrate how medium composition, salinity, and extraction parameters critically shape natural product discovery from extreme-environment *Streptomyces*.

A096

Investigating T. claveryi and T. boudieri and boudieri Extract as an Innovative Strategy to Address Methicillin-resistant Staphylococcus aureus by Mitigating Antimicrobial Resistance

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Abstract

The escalating global challenge of methicillin-resistant Staphylococcus aureus (MRSA) demands innovative therapeutic approaches. This study aims to explore the antibacterial and antibiofilm properties of two species of truffles, Terfezia claveryi and Terfezia boudieri extracts against both Staphylococcus aureus and Methicillin-resistant Staphylococcus aureus and to determine their mechanisms of action. The extracts' antibacterial and antibiofilm activities will be assessed against methicillin-resistant Staphylococcus aureus in vitro and their cytotoxicity will be evaluated on epithelial cells to ensure safety. Mechanistic insights will be explored using untargeted metabolomics. The extracts are expected to exhibit differential bactericidal activity against Staphylococcus aureus and Methicillin-resistant Staphylococcus aureus. A multi-passage resistance assay will be performed to evaluate potential resistance to the extracts over repeated exposure. Untargeted metabolomics will be used to annotate the metabolites and uncover the mechanism by which the Terfezia extracts elicit their antimicrobial effect. This study will ultimately uncover Terfezia species extracts as a promising new antibacterial and antibiofilm agent with potential as an alternative treatment for biofilm-related infections caused by S. aureus and multidrug-resistant Staphylococcus species.

A097

Investigating How *Pseudomonas aeruginosa* Escapes Predation by *Bdellovibrio bacteriovorus*

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Abstract

Bdellovibrio bacteriovorus is a predatory bacterium with potential as a "living antibiotic" due to its ability to kill Gram-negative pathogens. Realising this therapeutic promise requires understanding how prey species evade predation. *Pseudomonas aeruginosa*, a multidrug-resistant pathogen, is notably recalcitrant to predation by *B. bacteriovorus*, yet the mechanisms underlying this defence remain unexplored.

In this study, we examined components secreted by *P. aeruginosa* PA14 for their capacity to inhibit predation by *B. bacteriovorus*. Preparations from PA14 spent culture media (minimal M9 medium), enriched by ammonium sulphate precipitation, completely inhibited predation and rapidly killed *B. bacteriovorus* without affecting the viability of other Gram-negative bacteria, suggesting a highly specific mechanism of action. Treatment of these preparations with proteinase K or heating at 95^{o}C for 10 minutes abolished the anti-Bdellovibrio activity of the preparations, suggesting the active component was proteinaceous.

Subsequent fractionation of active preparations using anion-exchange and size-exclusion chromatography yielded fractions exhibiting strong anti-Bdellovibrio activity. Proteomic analysis via LC-MS/MS identified the presence of a single protein, the *P. aeruginosa* virulence factor protease-IV, encoded by the piv/prpL gene. Recombinant expression and purification of His-tagged protease-IV confirmed the anti-Bdellovibrio activity of this protein via reduction of predator viability measured by PFU/mL. Fluorescent microscopy using mCherry tagged *B. bacteriovorus* and live/dead staining suggests protease-IV mediated cell death by *B. bacteriovorus* membrane damage. Ongoing work to delete piv/prpL from the genome of *P. aeruginosa* will elucidate the contribution of this serine-protease to the escape of *P. aeruginosa* from predation by *B. bacteriovorus*.

A098

From Rare to Rising: Two Decades of Increasing Plasmid-Mediated Fluoroquinolone Resistance in Chickens

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Abstract

Fluoroquinolones are important antibiotics for human and animal health. Despite a 93.1% reduction in the sales of veterinary fluoroquinolones in the Netherlands since 2011, resistance remains common in indicator *Escherichia coli* from chicken caecal samples (25.6% in 2023). Fluoroquinolone resistance is caused by chromosomal mutations and plasmid-mediated genes. Plasmids play a pivotal role in the dissemination of antibiotic resistance, posing a serious public health risk. This study investigated the prevalence and genetic characteristics of plasmid-mediated fluoroquinolone resistance (PMQR) in *E. coli* isolated from chickens in the last two decades.

E. coli isolates from the Dutch routine antimicrobial resistance monitoring of chickens were used in this study. Isolates resistant to ciprofloxacin (>0.06 mg/L, EUCAST epidemiological cutoff) collected between 2004 and 2023 (n=2,568) were screened by PCR for PMQR genes. PMQR-positive isolates were sequenced using combined long-read and short-read methods.

Among ciprofloxacin-resistant isolates, the prevalence of PMQR increased from 0.4% in 2008 to 10.8% in 2023. Analysis shows the isolates belong to diverse sequence types, with ST752 and ST189 predominating. The most frequently detected PMQR genes were *qnrS1* and *qnrB19*. All *qnrB19* genes were found on Col plasmids, while *qnrS1* was found on a variety of plasmid types, most commonly IncX. bla_{TEM} genes frequently co-occurred with *qnrS1* on these plasmids.

This research demonstrates a substantial increase in PMQR over 20 years in chickens, despite reductions in fluoroquinolone use. The increase in plasmid-mediated resistance is particularly concerning given their capacity to spread between bacterial species and across environmental, animal and human reservoirs.

A099

Detection and Characterisation of Carbapenemase-Producing Enterobacteriaceae (CPE) in the Faeces of Irish Cattle

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Abstract

The rise of AMR poses a growing threat to human, animal, and environmental health. CPE are of particular concern as they are resistant to carbapenems, critically important antibiotics used to treat MDR and ESBL infections. Detection of CPE in bovine faeces suggests a potential environmental reservoir and risk of horizontal gene transfer of carbapenemase genes which can disseminate across bacterial species.

This study aimed to detect and characterise CPE in bovine faecal samples through phenotypic, molecular, and proteomic analyses. Bovine faecal samples (n= 450) were screened using selective media and confirmatory carbapenemase detection tests. Phenotypic testing identified CPE in 13.7% or 62 (n = 450) of samples. Of these, 20 CPE isolates were selected for further analysis. MALDI-TOF MS STAR was used to identify hydrolysis of the b-lactam ring and polymerase chain reaction (PCR) with Sanger sequencing was employed to detect *CPE* genes. MALDI-TOF MS identified hydrolysis of carbapenems consistent with CPE in 45% (n = 20) of isolates, while PCR detected the CPE gene *blaVIM* in 6.25% (n = 16) of isolates. The discrepancy between results was observed due to suspected loss of resistance in the isolates over time. The bacteria once isolated from faeces did not display resistance for an extended period of time.

The detection of CPE genes in Irish cattle confirms the presence of clinically significant resistance in agricultural environments. These findings highlight the need for enhanced One Health surveillance, biosecurity measures, and antimicrobial stewardship to mitigate the spread of CPE and protect public health.

A100

Responses of Clostridioides difficile, Bacillus subtilis, and Klebsiella pneumoniae to Sodium Hypochlorite Biocide Exposure

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Abstract

The widespread use of oxidising biocides in healthcare environments exerts strong selective pressure on microorganisms, yet the adaptive responses of clinically relevant bacteria such as *Clostridioides difficile* to repeated exposure remain poorly understood. This project investigates the evolutionary adaptation of spore-forming bacteria (*Clostridioides difficile* and *Bacillus subtilis*) and a Gram-negative pathogen (*Klebsiella pneumoniae*) to one commonly used oxidising agent, sodium hypochlorite (NaOCl). To assess adaptive changes in tolerance and survival, each species was serially passaged over multiple generations with sub-lethal biocide concentrations, mimicking exposure healthcare settings. Experimental approaches used to evaluate phenotypic changes included enumeration assays, minimum inhibitory and bactericidal concentration (MIC/MBC) testing, and spore germination assays. Comparative analyses sought to identify trends across species in biocide tolerance and spore resilience, and adaptive mechanisms using SEM and whole genome sequencing. The outcomes of this pilot study shed light on how repeated oxidising biocide exposure and the overuse of antimicrobial agents influences bacterial persistence and resistance to antimicrobials, thus contributing to improved understanding of disinfection efficacy and microbial adaptation in healthcare settings.

A101

Responses of *Clostridioides difficile*, *Bacillus subtilis*, and *Escherichia coli* to Sodium dichloroisocyanurate Biocide Exposure

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Abstract

Antimicrobial-Resistant (AMR) bacteria are a significant global healthcare challenge, leading to increased incidence of infection globally. These bacteria can adapt and respond to varying antimicrobial agents including varying biocides used in disinfection. This pilot study sought to assess the efficacy of sub-lethal concentrations of oxidising biocide Sodium dichloroisocyanurate (NaDCC) against *Clostridioides difficile, Bacillus subtilis, and Escherichia coli* via serial passage. To assess adaptive changes in tolerance and survival, each species was serially passaged over multiple generations with sub-lethal biocide concentrations, mimicking exposure healthcare settings. Experimental approaches used to evaluate phenotypic changes included enumeration assays, minimum inhibitory and bactericidal concentration (MIC/MBC) testing, and spore germination assays. Scanning electron microscopy and whole-genome sequencing were used to assess phenotypic and genomic changes after serial biocide exposure. This study aimed to determine whether these strains adapted to sub-lethal biocide use, emphasizing the importance of strict disinfection procedures to prevent resistance from emerging. These findings have implications for effective disinfection of clinical environments and understanding microbial adaptation in response to biocides.

A102

Antimicrobial Prescribing Practices for Surgical Prophylaxis in Caesarean Sections at Kawempe National Referral Hospital, Kampala, Uganda

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Abstract

Antimicrobial resistance (AMR) is a global public health threat, especially in developing countries, where limited antimicrobial stewardship programs contribute to inconsistent prescribing practices for Caesarean-section (CS) surgical prophylaxis. This study investigated physicians' knowledge and prescribing practices related to CS surgical antibiotic prophylaxis at Kawempe National Referral Hospital (KNRH) in Uganda. An anonymous electronic survey was conducted among 200 prescribing healthcare providers involved in CS procedures. The survey assessed demographic characteristics, knowledge of AMR, stewardship practices, and adherence to CS antibiotic prophylaxis guidelines. Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 27, with a p value < 0.05 considered statistically significant. The most chosen antibiotic was ceftriaxone, followed by metronidazole, either alone or in combination, both of which are not recommended according to World Health Organization (WHO) guidelines for CS prophylaxis. Most antibiotics were administered 15 to 60 minutes prior to surgery, which is also not in alignment with recommendations. Overall, the study revealed limited compliance with national and international guidelines on antibiotic prescription for CS prophylaxis among healthcare professionals, particularly regarding antibiotic choice, timing of administration, frequency, and dosage. The study reveals major gaps between recommended and actual antimicrobial prophylaxis practices for CS at KNRH. Despite clinicians' awareness of AMR, systemic barriers such as poor microbiological testing and reliance on peer advice hinder adherence. These findings reflect on the importance of policy development and the implementation of antimicrobial stewardship programs in Uganda to improve prescribing practices among healthcare providers.

A103

Genomic and Phenotypic Insights into the Persistence of Fluoroquinolone Resistant *Campylobacter jejuni*

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Abstract

Campylobacter jejuni is a major cause of bacterial gastroenteritis. Fluoroquinolone resistance in *C. jejuni* continues to persist despite policies restricting the use of fluoroquinolones in agriculture. Resistance in *C. jejuni* typically arises through mutations in the *gyrA* gene, which have been shown to alter DNA supercoiling, influencing a variety of phenotypes, including motility, biofilm formation, and virulence. To investigate the phenotypic consequences of fluoroquinolone resistance, two laboratory strains and two recently isolated strains of *C. jejuni* were gradually exposed to increasing concentrations of ciprofloxacin. This yielded isolates with low (≤10 µg/mL), intermediate (≤40 µg/mL), and high (>40 µg/mL) resistance levels. PCR confirmed the T86I substitution in *gyrA* across all resistant isolates. Motility assessed by the soft agar method showed strain-specific variations between resistant and susceptible isolates. Biofilm formation, visualized by fluorescence microscopy under both microaerobic and aerobic conditions, revealed changes in structure and viability of biofilms formed by resistant isolates. Additional assays showed changes in secreted protein profile, aerotolerance, and DNA supercoiling. Whole-genome sequencing of the isolates identified shared SNPs associated with these phenotypes. These results demonstrate that fluoroquinolone resistance in *C. jejuni* is accompanied by shifts in multiple phenotypes, which may enhance survival, transmission, and virulence.

A104

Quantifying intrinsic and acquired antimicrobial resistance in poultry microbiomes through integrated MAG-context and 16S rRNA- AMR AmpliSeq normalised analysis

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Abstract

Antimicrobial resistance (AMR) in livestock microbiomes reflects a complex interplay between intrinsic genetic traits and acquired resistance shaped by antimicrobial use and horizontal gene transfer. Distinguishing between these processes is essential for interpreting surveillance data and identifying microbiomes under selection pressure.

Here, we combine metagenome-assembled genomes (MAGs) with paired 16S rRNA-AMR AmpliSeq profiles to classify resistance genes and microbiome samples along an 'intrinsic-acquired' continuum, using normalised taxon-ARG ratios and mobility priors learned from shotgun MAG and plasmid data. Using hybrid assemblies from poultry caecal metagenomes, antimicrobial resistance genes (ARGs) were mapped to their genomic context and scored for chromosomal integration, copy number, and plasmid association. This produced gene-level mobility scores distinguishing single-copy, chromosomally integrated loci from multicopy or plasmid-borne genes. Parallel 16S rRNA and AMR AmpliSeq-based genome-equivalent normalisation allowed ARG abundances to be scaled to total bacterial load and species richness, enabling inter-sample comparisons independent of sequencing depth.

By integrating these layers, we derived a sample-level *resistance acquisition index* reflecting the proportion of mobile, multicopy ARGs relative to total taxonomic diversity. Samples dominated by intrinsic, single-copy ARGs exhibited stable baseline resistomes, whereas those enriched in plasmid-mediated or multicopy ARGs corresponded to evidence of antimicrobial exposure or selective pressure.

This approach bridges genome-resolved and community-wide AMR analyses to quantify resistome structure and risk. The resulting framework provides a scalable means to identify microbiomes under selection, benchmark background resistance, and enhance surveillance sensitivity across agricultural and clinical settings.

A105

Pairwise co-cultures reveal antibiotic- and partner-dependent growth and gene expression in the presence of a multi-resistant *Escherichia coli*

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Abstract

Background

Subinhibitory antibiotic concentrations are frequently encountered in the intestinal environment, where gradients, inoculum effects and community-level interactions modulate antimicrobial activity. *Escherichia coli*, a minor constituent of the healthy gut microbiota, can expand significantly during dysbiosis or antibiotic exposure and has been co-isolated with other *Enterobacterales* in polymicrobial infections. Here, we systematically investigated pairwise interactions between a multidrug-resistant clinical *E. coli* IMM939 and representative commensal or opportunistic gut bacteria, quantifying shifts in growth dynamics during antibiotic stress.

Methods

Using dual-well microplates, allowing metabolite exchange without cell contact, *E. coli IMM939* was co-cultured anaerobically with twelve partner strains (five commensals, seven pathogens) in the absence or presence of ciprofloxacin (0.032 µg/mL) or piperacillin/tazobactam (1 µg/mL; 4 µg/mL).

Results

Across the tested panel, cooperative effects were most pronounced among *Enterobacterales* partners under ciprofloxacin exposure. In fact, all three *Enterobacterales* partners (*Enterobacter ludwigii* IMM005, *Enterobacter cloacae* DSM30054 and *E. coli* ATCC25922) showed increased biomass in co-culture with *E. coli* IMM939 compared with their monocultures under ciprofloxacin stress ($\log_{10}(AUC_{(co\text{-}culture)}/AUC_{(mono)})$: *E. ludwigii* = 0.25, *E. cloacae* = 0.21, *E. coli* ATCC 25922 = 0.72; n = 3). Despite susceptibility (MIC 0.016 µg/mL), *E. coli* ATCC 25922 exhibited elevated biomass in co-culture compared with its negligible growth in monoculture. However, these effects were not observed with piperacillin/tazobactam (4 µg/mL).

The findings indicate that drug-specific subinhibitory antibiotic conditions may foster cooperative protection among *Enterobacterales*, which could contribute to their disproportionate expansion and persistence during antibiotic-induced dysbiosis.

A106

Nationwide Analysis of Antimicrobial Resistance in Soil Environments to Bridge the Current Data Knowledge Gaps

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Abstract

Introduction

The World Health Organization (WHO) has identified antimicrobial resistance (AMR) as one of the greatest global threats to human health. To combat this, it recommends intensive monitoring to identify and track critical AMR "hotspots" across the environment, humans, animals, and the pathways connecting them.

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

Currently, there is no surveillance of AMR in global agricultural soils, leaving a major knowledge gap. This project aims to address that gap through a nationwide survey of AMR in 3,000 Irish agricultural soil samples collected as part of the DAFM Farm Environmental Study Programme.

Method

From these soil samples, and using high throughput processes, DNA was extracted to create a national soil and DNA biobank, and all DNA extracts were screened by qPCR for several clinically relevant AMR genes, including those responsible for extended-spectrum β-lactamases (ESBLs) and carbapenemases.

Results

Initial results indicate that *sul1* and *intl1* were nearly ubiquitous in the samples, detected in 88% and 86% of soils, respectively. In contrast, *blaKPC* and *blaVIM* were virtually absent, occurring at only 0.27% and 0.1% respectively.

Conclusion

This work provides the first comprehensive overview of AMR in Irish agricultural soils, delivering essential data to inform local, national, and EU-level policies aimed at reducing the spread of antimicrobial resistance.

A107

A student-led network: NextGenAMR

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Abstract

Antimicrobial resistance (AMR) is a complex and cross-sectoral issue, requiring multi-, inter-, and transdisciplinary collaboration. Yet, in the UK, students and early-career researchers (ECR's) often lack spaces to connect or engage across disciplines concerning AMR, and ECR's struggle to share their work beyond academia. Here we present NextGenAMR, a student-led network co-founded by two EastBio DTP PhD students, providing an open, supportive platform for emerging AMR researchers. Grounded in a One Health approach, NextGenAMR connects students and ECR's, facilitating collaboration across disciplines and institutions, with the aim of driving conversations within academia and to wider audiences. Since launching in July 2025, NextGenAMR has hosted meetings and webinars, participated in public outreach actions and created social media content, such as a short film for World Antimicrobial Resistance Awareness Week 2025. Members include both AMR researchers and those from related fields, including animal welfare and molecular epidemiology, reflecting the network's holistic outlook. Looking ahead, we aim to expand our network and lead more engaging events with interested parties, involving a broad array of stakeholders that play a direct role in decreasing AMR's impact. Our mission is to help members to engage with different facets of the AMR problem, and practice and enhance scientific communication, so that research remains accessible and relatable. Ultimately, NextGenAMR prepares the next generation to address AMR innovatively while strengthening skills in collaboration, leadership, and public engagement.

A108

Sodium Cyclamate can inhibit pathogen growth and overcome native antibiotic resistance mechanisms.

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Abstract

Antimicrobial resistance is escalating globally, with projections estimating up to 40 million deaths by 2050. The rise of multidrug-resistant pathogens, coupled with a stall in antibiotic development, creates immense pressure on the scientific field to develop new antimicrobial treatments. Our previous research revealed that artificial sweeteners can influence bacterial behaviour. Building on this, we investigated the effects of sodium cyclamate on the morphology of *Escherichia coli*. Exposure to sodium cyclamate resulted in cell elongation, membrane bulging, and lysis, suggesting an interference with the septation and DNA replication systems. Using DNA replication reporters, we could temporally visualise and quantify this impact on DNA replication dynamics. Based on this, we next sought to assess sodium cyclamates' broader antimicrobial potential by evaluating its impact on a panel of multidrug-resistant strains, observing selective efficacy with *Acinetobacter baumannii* being particularly sensitive. To explore the mechanism, we conducted RNA-Seq analysis, which revealed a range of pathways linked to virulence that were impacted. Given the impact on *E. coli* morphology and the associated transcriptional signatures, we wanted to investigate whether cell morphology changes also occur in *A. baumannii*. Live cell imaging revealed morphological changes and increased membrane permeability that also led to a concomitant increase in sensitivity to a range of antibiotics, including carbapenems. To explore the translational potential of these findings, we tested sodium cyclamate-loaded wound dressings, demonstrating reduced bacterial viability in ex vivo models. These findings highlight the potential of sodium cyclamate as both an antimicrobial therapy and a potentiator for currently available antibiotic treatments.

A109

A Novel Host Depletion Method for Diagnosis of Drug-Resistant Bacterial Infections using Clinical Metagenomics

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Abstract

Bacterial infections can be challenging to diagnose through traditional culture-based methods due to slow growing or fastidious bacteria. Importantly, delays in diagnosis, or an inability to identify the causative pathogen, can lead to inappropriate antibiotic use. Metagenomic whole-genome sequencing has become increasingly accessible in clinical settings, offering a rapid, culture-independent alternative for predicting species and antibiotic sensitivity profile. However, samples with low bacterial load or high numbers of host cells can limit sensitivity, requiring effective host depletion methods to increase the proportion of microbial reads and the likelihood of an accurate diagnosis.

Typical host depletion protocols involve selective lysis of host cells using osmotic shock or non-ionic detergents, followed by removal of cell debris and incubation with non-specific endonucleases to degrade free-floating DNA. The intact bacterial cells are then lysed and undergo extraction for metagenomic sequencing. We have developed a novel host depletion method to improve the workflow for isolating bacterial DNA from clinical samples. Following bead-beating to selectively lyse host cells, magnetic beads are used to bind and pellet the released host DNA. The bacterial cells remain suspended in the supernatant, from which DNA is then purified for downstream analysis. Our preliminary testing using spiked samples shows a 99.99% reduction in host DNA when quantified using qPCR.

This method does not require specialised enzymes and avoids prolonged incubation periods at increased temperatures or incomplete digestion by endonucleases, overall facilitating a faster workflow, reducing the time between sampling results and leading to more rapid and sensitive diagnostics.

A110

Dynamics of AMR selection in *Campylobacter* under photodynamic stress

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Abstract

AMR in *Campylobacter* threatens effective therapy, with fluoroquinolone and tetracycline resistance remaining common despite curtailed use in UK poultry, *Campylobacter*'s primary host, indicating low fitness costs and persistence of resistance determinants. In UK *C. jejuni* isolates, multidrug resistance (MDR) is rare (~0.8%), but very high burdens are reported elsewhere. In other agricultural reservoirs, for instance *C. coli* from pigs, MDR is more common, underscoring continued selection risk across the food chain. Violet-blue photodynamic inactivation (VB-PDI) offers a non-antibiotic intervention by exciting endogenous porphyrins to generate reactive oxygen species (ROS) that damage multiple essential cellular targets, leading to cell death. Recently, we assessed the interaction between AMR status and VB-PDI in 65 diverse *Campylobacter* isolates. All were susceptible to VB-PDI regardless of AMR genotype or phenotype. We conducted an initial laboratory evolution experiment with repeat exposure to VB-PDI, revealing no increase in tolerance or persister population-like survival after bactericidal dosing. While these data indicate a low propensity for resistance evolution directly to VB-PDI, we did consider that co-selection for AMR may occur under VB-PDI stress, as has been documented for some chemical biocides where sublethal exposure can enhance cross-resistance to antibiotics. It is also likely that *Campylobacter* in the food chain will be exposed to subinhibitory antibiotics from prior livestock prophylaxis, further increasing the pressure for AMR emergence. Here, we describe co-selection laboratory evolution experiments with *Campylobacter* stressed with VB-PDI and antibiotics. We model AMR emergence rates, and stratify the risk from non-antibiotic stressors.

A111

Mitigating MRSA Resistance: AHA-1394 as a Potential Polyamine-Derived Antimicrobial Compound

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Abstract

The increased trends of methicillin-resistant *Staphylococcus aureus* (MRSA) developing resistance to last-resort antibiotics create the urgent need to develop a novel antimicrobial compound. Our synthetic polyamine, AHA-1394, shows promising potential against MRSA in both *in vitro* and *in vivo* settings.

We used minimum inhibitory concentration (MIC), time-kill kinetic assays, and checkerboard analyses, to determine antibacterial efficacy and potential synergy between AHA-1394 and clinically relevant antibiotics. We expanded our investigation to evaluate its drug-like properties, such as inoculum effect, interaction with human serum albumin (HSA), and post-antibiotic effect. *In vivo* efficacy was tested using the *Galleria mellonella* larval injection model.

Results show that AHA-1394 is bactericidal across a broad range of *S. aureus* strains. Time-kill kinetic assays confirm its time-dependent and synergistic activity with existing antibiotics, especially β-lactams. A two-fold increase in MIC was observed with higher inoculum size. Importantly, AHA-1394 is not affected by the presence of HSA and it exhibits an 8-to-10-hour post-antibiotic effect, indicating its potential for less frequent dosing. In an *in vivo* setting, AHA-1394 significantly increases larval survival, supporting its therapeutic potential.

Our study highlights AHA-1394 as a promising candidate to treat staphylococcal infections, including strains resistant to last-line antibiotics. Its synergy with other antibiotics may enhance efficacy and help prevent the development of further resistance. With favourable drug-like properties, AHA-1394 stands out as a novel antimicrobial compound with clinical significance as both a standalone and adjunctive therapy in addressing antimicrobial resistance.

A112

In Vitro Evaluation of the Combined Effects of Meropenem and Peppermint Essential Oil on New Delhi Metallo-Beta-Lactamase (NDM)-Producing *Escherichia coli*

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Abstract

Objective: This study aimed to determine the minimal inhibitory concentration (MIC) of meropenem and peppermint essential oil and to assess their combined effect against New Delhi metallo-beta-lactamase (NDM)-producing *Escherichia coli* isolated from a public hospital in Hong Kong.

Methods: The MIC was determined using broth micro-dilution according to the Clinical and Laboratory Standards Institute (CLSI) M07 12th edition. Peppermint oil was prepared in dimethyl sulfoxide and diluted in cation-adjusted Mueller-Hinton broth containing 1% Tween 80. The in vitro effects of the antibiotic combined with essential oils against bacterial isolates were assessed using the checkerboard assay. Results were expressed as the fractional inhibitory concentration index (FICI). Synergy was defined as a FICI ≤ 0.5, indifference as a FICI between >0.5 and 4, and antagonism as a FICI > 4.

Results: Among the fourteen isolates tested, MIC values for meropenem ranged from 8 to >16 μg/mL, while those for peppermint oil ranged from 8 to >32 mg/mL. The FICI for the combination of meropenem and peppermint oil in thirteen strains ranged from 0.13 to 0.50, indicating synergy, whereas one strain showed indifferent interaction (FICI of 1.00).

Conclusions: This study demonstrated that the combination of peppermint essential oil and meropenem exhibited in vitro synergistic effects against NDM-producing *Escherichia coli* in 92.9% of cases. Our findings provide an alternative strategy for addressing the significant challenge posed by these resistant bacterial strains.

A113

Evaluation of In Vitro Combination Effects Between Gepotidacin and Clove Essential Oil on Klebsiella pneumoniae Carbapenemase (KPC)-Producing *Klebsiella pneumoniae*

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Abstract

Objective: This study aimed to determine the minimal inhibitory concentration (MIC) of gepotidacin and clove essential oil, and to assess their combined effect against multi-drug resistant *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* isolated from a public hospital in Hong Kong.

Methods: The MIC was determined using broth micro-dilution according to the Clinical and Laboratory Standards Institute (CLSI) M07 12th edition. Clove oil was prepared in dimethyl sulfoxide and diluted in cation-adjusted Mueller-Hinton broth containing 1% Tween 80. The in vitro effects of the antibiotic combined with essential oils against bacterial isolates were assessed using the checkerboard assay. Results were expressed as the fractional inhibitory concentration index (FICI). Synergy was defined as a FICI ≤ 0.5, indifference as a FICI between >0.5 and 4, and antagonism as a FICI > 4.

Results: Among the ten isolates tested, MIC values for gepotidacin ranged from 4 to >16 µg/mL, while those for clove oil ranged from 8 to 16 mg/mL. A synergistic effect was observed in the combination of gepotidacin and clove oil against two KPC-producing *Klebsiella pneumoniae* strains, both showing FICI values of 0.5. Other strains exhibited indifferent interactions, with FICI values ranging from 0.625 to 0.75.

Conclusions: This study demonstrated in vitro synergistic effects of clove essential oil combined with gepotidacin against KPC-producing *Klebsiella pneumoniae* in 20% of cases. This novel combination offers an alternative strategy for addressing the critical challenge posed by these multi-drug resistant KPC-producing strains.

A114

The Impact of Maternal Vaccination on the Establishment of the Microbiome in the Neonate.

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Abstract

The first four weeks of life are crucial for a baby's healthy development. Microbial and immunological exposures during the neonatal period shape long-term health outcomes and disease risk. As newborns have an immature immune system and an underdeveloped microbiome, they are highly susceptible to contracting bacterial infections. Such infections can rapidly develop into sepsis, a leading cause of infant mortality worldwide. Neonatal sepsis disproportionately affects low- and middle-income countries where Gram-negative pathogens are the most common cause. While antibiotics are the primary treatment, rising antimicrobial resistance (AMR) underscores the need for alternative therapies and preventative measures.

Vaccination is a promising preventative strategy to combat AMR. Previous work within our laboratory has identified candidates capable of providing cross-protective immunity to offspring born to vaccinated female mice. Protection is conferred against *Klebsiella pneumoniae* and *Enterobacter spp.*, two WHO priority pathogens. As the candidates are highly conserved, their impact on other microbiota, particularly beneficial bacteria, present in the establishing neonatal microbiome needs to be determined. Given the critical interplay between the immune system and microbiome during early life, it is essential to assess the candidates' impact on neonatal microbiome establishment.

The impact of several vaccine candidates on the gut, skin, and lung microbiomes of vaccinated dams and offspring was investigated using maternal immunisation murine models and microbial culturing techniques. Preliminary results showed no impact of vaccination on the gut microbiome. Subsequent studies will explore impact further using molecular and histological techniques, expanding to immunological analyses to uncover more about the candidates' immunogenic potential.

A115

Mechanistic killing of bacteria on nanostructured surfaces

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Abstract

Bacterial colonisation of surfaces and antibiotic resistance are some of the biggest threats to global human health. Nanostructured surfaces (NSS) could provide a solution to both problems simultaneously through their bactericidal properties. Previous work has proven that the bacterial killing process of NSS is largely mechanistic, making them broadly effective and likely insusceptible to resistance over time. Furthermore, eukaryotic cells are not susceptible to the killing effect of NSS, so there is potential for their use on internal medical devices and other clinical applications. However, there are many challenges to the design and testing of NSS, including surface biofouling by dead cells and specificity to microbial size/shape [1]. Most importantly, we do not understand how bacterial pathogens are killed by NSS. My research aims to uncover the NSS killing mechanism and optimise its efficiency for use in clinical or other settings. We initially carry out a comparative study to compare the effect of each NSS parameter (height, width, pitch, shape and stiffness, assessed with AFM/SEM), addressing a lack of standardisation across the literature. Killing efficacy is assessed through Live/Dead staining in combination with fluorescence microscopy, as outlined in [2]. The NSS killing mechanism is investigated using a combination of optical tweezers and super-resolution microscopy. This involves placing individual cells with distinctively-dyed layers onto NSS and observing the killing process. Through this research we hope to address the lack of consensus across previous studies regarding NSS parameters' effects on killing efficacy and the mechanism behind the killing effect.

A116

Detection of viable cells following antibiotic treatment using TTC has potential for low-cost, rapid detection tests for antimicrobial resistance

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Abstract

The rapid and reliable detection of antimicrobial resistance (AMR) in multidrug-resistant pathogens remains one of the most urgent challenges in clinical microbiology. Delays in diagnosis contribute to inappropriate treatment, overuse of expensive antibiotics and poor patient outcomes. The use of triphenyltetrazolium chloride (TTC) as a colorimetric viability indicator for bacteria well known. We adapted this for detecting antibiotic resistance in multidrug resistant pathogens with the goal of developing a rapid and cost-effective alternative to conventional culture-based methods.

The research focused on optimising a metabolic activity assay using 2,3,5-triphenyltetrazolium chloride (TTC) to detect bacterial growth inhibition following antibiotic treatment. The project systematically investigated various experimental conditions to reduce the assay's detection time, including pre-warming materials, shaking for increased oxygenation, and optimizing bacterial cell density inputs. These efforts successfully reduced the initial detection time of over 12 hours to as little as 90 minutes from the addition of a standardised culture suspension.

The optimised assay was then validated through two distinct approaches. Firstly, results of the TTC rapid test were compared to results of a standardised EUCAST disc diffusion method for two clinically relevant wound pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (an MRSA strain). Secondly, the assay was used to differentiate between ampicillin resistant and susceptible strains of *S. aureus*. The results from both validation steps demonstrated the system's ability to accurately differentiate between susceptible and resistant bacterial strains in a way consistent to EUCAST disc diffusion. This shows promising prospects for future low cost, rapid and simple diagnostics for AMR.

A117

D-amino acids : the role of D-leucine in biofilm eradication

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Abstract

Lower respiratory tract (LRT) infections commonly involve persistent biofilms which contribute to chronic conditions and AMR. Recent research shows that amino acids are able to enhance drug solubility and disrupt biofilm components making them promising adjuvants.

In this study, D-leucine has been evaluated for its anti-biofilm activity against mono and polymicrobial biofilms of *P. aeruginosa* and *S. aureus*. Monomicrobial and polymicrobial biofilms were tested with D-leucine and amikacin using minimum inhibitory concentration (MIC), chequerboard assay, crystal violet staining. Biofilm structure and viability was further assessed with the help of confocal fluorescence live/dead assay imaging technique and ATP based luminescence assays.

D-leucine significantly inhibited biofilm formation by up to 63.3% biomass reduction and 58% reduction in thickness at 40mM compared to the untreated controls. In combination with amikacin, D-leucine showed synergy (FICI index ≤0.5), reducing the effective concentrations by 2-4-fold. The cell viability assays confirmed up to an 80% reduction in CFU values and a 20% decrease in ATP production in the treated samples in relative to the control.

The observed anti-biofilm activity may result due to D-leucine substituting L-leucine in leucine aminopeptidase, an enzyme essential for biofilm formation. Additionally, mutations in the dtd gene in *P. aeruginosa* could allow D-amino acids to be mistakenly incorporated in place of L-amino acids, further contributing to the antibiofilm effect. These hypotheses will be explored in future research utilising molecular techniques. Overall, these findings highlight D-leucine as a promising antibiotic adjuvant to combat biofilm associated AMR.

A118

Rhamnolipids as novel therapeutics for multi-drug resistant acne vulgaris.

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, Nigel Ternan ORCID iD

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Abstract

Acne vulgaris is a multifactorial disease which affects ~85% of adolescents and ~12% of adults in the UK. The condition is often recalcitrant to treatment due to antimicrobial resistant forms of the anaerobic skin bacterium *Cutibacterium acnes* that plays a role in the development of the inflammatory phase.

This work aimed to evaluate the antimicrobial effects of microbial rhamnolipids (RLs) against *C. acnes* using broth microdilution assays, checkerboard assays that examined RLs in combination with antibiotics and the antioxidant cosmetic, L-ascorbic acid (Vitamin C), as well as antibiofilm assays. Six bacterial isolates from various skin sources were utilised, all of which were confirmed to be phylotype IA_1 which are mostly acnegenic. Rhamnolipids were obtained from crude rhamnolipid mixture using a combination of liquid and solid phase extraction and the resulting samples confirmed by mass spectrometry analysis.

Mono-RL congeners (97.44%), di-RL congeners (98.72%) and mixed RL (67.51% di, 33.13% mono congeners) were all found to have antimicrobial activity, with MICs that ranged from 2.4 to 19 µg/ml. Checkerboard assays highlighted potential synergistic or additive effects when all three types of RL were combined with Vitamin C (Fractional inhibitory concentration index 0.37-0.75 and 0.5-4.5 with antibiotics). Antibiofilm effects of the RLs were also observed, with 1.3-15 fold reductions in treated biofilm quantity depending on the strain analysed. These results suggest that RLs have the potential to be a novel topical therapy for acne vulgaris.

A119

Harnessing the potential of trained immunity against AMR infections

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Abstract

New strategies are urgently needed to tackle the antimicrobial resistance (AMR) crisis. Bacterial infections are the second leading cause of death worldwide, with major pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* exhibiting widespread AMR. Trained innate immunity (TRIM) offers a promising approach by enhancing innate immune responses through epigenetic and metabolic reprogramming of hematopoietic stem cells, which is inherited by macrophages and neutrophils, the first responders to infection. Commensal bacteria, sharing recognition motifs with pathogens and displaying high diversity, represent a novel source of TRIM inducers to protect against AMR infections. Zebrafish infection models provide unparalleled insights into innate immunity, and we have recently pioneered this system to study TRIM mechanisms. Using this platform, we aim to systematically assess TRIM programs induced by commensal bacteria, potentially enabling innovative host-directed therapies that harness the body's own defences to control AMR infections.

A120

Characterisation of within-patient ciprofloxacin resistance single-nucleotide polymorphisms (SNPs) from clinical isolates of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa presents high levels of antimicrobial resistance in clinic and can develop resistance rapidly within-patient during treatment. Therefore, understanding the genetic mechanisms to detect and predict the emergence of this potential resistance has become a highly desirable goal. In previous studies we identified a range of SNPs that occurred *de novo* within the patient during treatment of *P. aeruginosa* infection with ciprofloxacin. The clinical strains which contain these SNPs presented diverse resistance levels or fitness cost. To disclose the real impact of these SNPs within-patient, a selection was introduced into the laboratory strain PAO1, which offered a standardized genetic background. Two techniques were used: The first consisted off a CRISPR-Cas toolkit that involves the combined action of the endonuclease Cas9 with a single stranded DNA recombinase (Ssr) which would introduce a single stranded DNA with the desired SNP in genomic DNA. The second was allelic exchange which consisted off a homologous recombination between a fragment of the gene with the desired SNP, cloned in a suicide vector, with the genomic DNA. Once we confirmed the modifications, studies of bacterial fitness were performed: growth rate in presence and absence of ciprofloxacin and minimum inhibitory concentration (MIC) in which the modified strains were compared with the parental PAO1 and clinical strains that contained the SNP. These studies will provide a better understanding of how the genetic background, that evolves differently in each patient, leads to diverse responses to antibiotic treatment during the infection in terms of resistance levels and fitness cost.

A121

Narrow spectrum drug repurposing for bacterial vaginosis.

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Abstract

Bacterial vaginosis (BV) affects 20–30% of females and is associated with significant adverse outcomes, including increased susceptibility to sexually transmitted infections and a higher risk of preterm birth. It is characterised by a reduction in beneficial *Lactobacillus* species and a concurrent overgrowth of anaerobic bacteria such as *Gardnerella vaginalis*. This dysbiosis promotes the formation of polymicrobial biofilms on the vaginal epithelium, contributing to recurrent infections. Recurrence is further complicated by the broad-spectrum nature of current antimicrobials, such as clindamycin.

We therefore aimed to employ a drug repurposing screen to identify novel pathogen-selective and antibiofilm molecules. Screening over 2,100 compounds identified several molecules with both inhibitory and antibiofilm activity against *G. vaginalis* but not against beneficial lactobacilli. Molecules of interest included an aminopeptidase N inhibitor, which inhibited biofilm metabolism at concentrations as low as 1.25 µM and demonstrated synergistic activity with the existing antimicrobial metronidazole (synergy score: 12.25).

Furthermore, ebselen, an organoselenium compound with known anti-inflammatory activity, showed significant activity against metronidazole-resistant strains and exhibited antibiofilm activity against a polymicrobial, four-species BV biofilm model. Further analysis of structural ebselen analogues improved the therapeutic selectivity window against lactobacilli and demonstrated no cytotoxicity towards VK2 epithelial cells at concentrations up to 80 µM.

To elucidate the cellular target of the compound, metatranscriptomic analysis was performed on the polymicrobial BV biofilm model at sub-inhibitory concentrations. These findings refine the list of lead compounds and reinforce drug repurposing as a viable strategy to address recurrent BV.

A122

Exploring *Bacillus subtilis* to induce trained immunity in zebrafish

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Abstract

Trained innate immunity (TRIM) is characterised by long-lasting, non-specific enhancement of innate immune responses upon infectious challenges. This protective effect is mediated by epigenetic and metabolic reprogramming of haematopoietic stem cells (HSCs) and myeloid cells, such as macrophages and neutrophils. Given the current state of antimicrobial resistance (AMR), new strategies are required to address this crisis. TRIM emerges as an innovative solution to combat infections and decrease antibiotic usage in clinical settings. The zebrafish embryo model has recently been demonstrated to be a powerful model for studying TRIM, as innate immune responses can be studied in isolation during early development, and their transparency, combined with fluorescent transgenics, allows for observation of immune reprogramming *in vivo*. Work on TRIM has mostly focused on Bacillus Calmette-Guérin (BCG) and the fungal wall component β-glucan; however, other bacterial TRIM inducers can be exploited. Here, we focus on the commensal bacteria *Bacillus subtilis* as a training inducer to protect against infections by AMR pathogens. Using the zebrafish TRIM model suggests that, during immune training, *B. subtilis* activates expression of the pro-inflammatory cytokines TNF-α and IL-1β. Similar to other TRIM inducers, training with this commensal bacteria elicits neutrophil production by 48 hours post-training, indicating that HSCs are responding to the stimulus. We have also observed that zebrafish embryos have improved survival following heterologous challenge with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Understanding how a safe commensal like *B. subtilis* reprograms innate immunity will inform the rational design of microbiota-based TRIM inducers for use against infection and AMR.

A123

AMR in the environment: a survey of bacterial resistance in urban water in northern Italy

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Abstract

Antimicrobial Resistance (AMR) has been officially recognised as one of the top 10 global health threats, with an estimated 4.95 million deaths associated with bacterial AMR in 2019 alone. In recent years, there has been a growing recognition of the need to address AMR through a One Health framework, which acknowledges the interconnectedness of human, animal, and environmental health. Despite this conceptual shift, the environmental component of the One Health approach remains comparatively understudied, particularly in urban aquatic systems, where human, agricultural, and industrial wastewaters converge. Rivers and canals in urban environments are increasingly recognised as critical conduits for AMR propagation, often containing mixtures of resistant bacteria, antibiotic residues, and mobile genetic elements that promote gene exchange. In this study, we investigated the prevalence and distribution of antimicrobial-resistant bacteria in a selection of peri urban water streams of Padova, a densely populated city in northeastern Italy. Through a preliminary survey based on a four-weeks period of weekly sampling at multiple sites, we isolated, sequenced and characterised drug-resistant bacteria from different water bodies. We identified *E. coli* as the most frequently isolated species, and species with the highest count of antimicrobial resistance. Genome analysis also revealed the potential for pathogenicity of some of the isolates, and the diversity of genes and mutations involved in resistance. This study illustrates the spread of AMR in the environment and highlights the need for efficient wastewater treatment to limit it.

A124

Mobile Resistance Genes in Archaea: Genomic Evidence and One Health Implications

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Abstract

Archaea and bacteria share a close phylogenetic relationship and are both ubiquitous environmental components that exchange functional genes. However, it remains unclear how this evolutionary proximity and gene flow influence the carriage of antimicrobial resistance (AMR) genes in archaea or their role in AMR development in co-localised bacterial pathogens. This study investigated the diversity and abundance of antimicrobial resistance genes (ARGs) in archaeal genomes to clarify their role in AMR transmission, particularly through horizontal gene transfer (HGT).

Archaeal genomes from the Genome Taxonomy Database (v2.4.1, release 220), including 10,740 metagenome-assembled (MAGs), 1,601 pure-culture (WGS) and 136 single-cell (SAG) genomes, were screened for ARGs using PanRes (v1.0.2). A total of 47 ARGs were identified in MAGs, 111 in WGS and one in SAG, predominantly distributed within *Methanobacteria*, *Halobacteria*, and *Thermoproteia,* typically isolated from host-associated samples, high-salinity waters or hot springs. Of these, 34% (MAGs) and 76.9% (WGS/SAG) were biocide or metal ARGs. Using mobileOG-db, 13 mobile genetic elements (MGEs) were identified adjacent to 16 ARGs across the dataset, suggesting potential HGT, particularly in strains from environmental or host-associated samples. Ongoing work involves validating potential HGT between archaea and bacteria through comparative genomic analyses and integrating biosample metadata to trace transfer pathways and ecological distributions, clarifying archaeal contributions to AMR transmission across domains of life.

This study highlights archaea as an overlooked but potentially significant reservoir of mobile ARGs within the One Health continuum, offering new insights into AMR gene exchange and strategies to mitigate global AMR risks.

A125

Developing a targeted metagenomic platform for surveillance of antibiotic resistance genes in environmental samples

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Abstract

Environmental surveillance of antibiotic resistance genes (ARGs) can aid in our understanding of ARG transfer and persistence between geographical regions and within ecosystems. Current surveillance methods rely on culturing isolates and multi-plex PCR, which can be labour-intensive and limited to a small number of clinically relevant pathogens. More recently, metagenomic sequencing methods are being employed to determine the total resistome of diverse environmental systems, which has the benefit of capturing resistance information from commensal bacteria as well as pathogens. However, metagenomic surveillance efforts are limited by the cost of obtaining sufficient sequencing depth to fully capture ARGs and there are no established criteria for assessing risk of environmental AMR from resistome data. To facilitate broad ARG surveillance at a reduced cost per sample, we have developed a targeted amplicon-based ARG platform (DARTE-QM) for use on environmental samples. Recognizing that environmental surveillance needs differ from clinical data needs, we designed the platform to target 379 genes considered high-risk of potential mobility into pathogens. We tested the platform on diverse samples including soil, groundwater, manure and wastewater. Samples show distinct resistance gene patterns according to source and reproducible diversity results between samples across runs. Compared to total resistome sequencing, DARTE-QM data is biased towards antibiotic inactivation mechanisms and against intrinsic genes such as membrane permeability and efflux pumps. Next steps are to correlate DARTE-QM data with culturing results and develop risk assessment models based on ARG detection.

A126

Genotypic evaluation of ESBL/AmpC producing- *E. coli* in health cattle in the UK

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Abstract

Genomic surveillance of antimicrobial resistance (AMR) in healthy animals ensures catching early trends in antimicrobial resistance gene (ARG) status, protects the food chain and safeguards antibiotic usage. As part of the Pathogen Surveillance in Agriculture, Food and Environment (PATH-SAFE) programme, we aimed to evaluate ARGs in *Escherichia coli* isolated from caecal content samples from healthy beef cattle (n=361). Sampling took place at slaughter across Great Britain for 8 months (2023/2024). Ninety-seven presumptive extended spectrum beta-lactamase (ESBL/AmpC) producing- *E. coli* were cultivated in ESBL selective media and classified phenotypically by Minimum Inhibitory Concentration. Whole genomes were generated and evaluated for ARGs using AMRFinderPlus (v.4.0.3). All strains phenotypically classified as ESBL (n=67) harboured a $bla_{\text{CTX-M}}$ gene, with 79.1% (53/67) of these being $bla_{\text{CTX-M-15}}$. In one third (69.2%; 18/26) of phenotypically AmpC strains (26.8%; 26/97), point mutation C-42T in AmpC promoter gene were detected. $bla_{\text{CMY-2}}$ (19.2%; 5/26) and $bla_{\text{DHA-1}}$ (3.8%; 1/26) were also described. Of the AmpC encoding strains, 11.5% (3/26) also contain bla_{TEM}, TEM-35 and CTX-M-15 genes, despite not being detected by phenotyping. Four strains were classified as ESBL and AmpC but genotypically, two harboured only $bla_{\text{CTX-M-15}}$ and two harboured $bla_{\text{DHA-1}}$ and C-42T in promoter AmpC gene. Among all strains sequenced, 46.3% (45/97) were multidrug resistant, with ARGs against quinolones (*65*), tetracycline (*36*) and aminoglycosides (*31*) more frequently found. Resistance against biocide agents was identified in 12.3% (12/97). Genomic analysis enhances the depth of research and surveillance and understanding the AMR landscape.

A127

Perspectives from reimbursors on the requirements of an inhaled combination drug including Disperazol to improve management of *Pseudomonas aeruginosa* infections in Cystic Fibrosis

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Abstract

Introduction: Lung disease resulting in chronic airway infection and inflammation is frequently cited as being the chief cause of both morbidity and mortality in Cystic Fibrosis with *Pseudomonas aeruginosa* the most common pathogen in a CF lung. A new antimicrobial such as Disperazol may be used to reduce chronic infection, provided it does not add to the patient's daily treatment burden. Disperazol disperses biofilms and reduces the required dose of Standard of Care antibiotics 100-fold to treat *P.aeruginosa* infections

Methods: The objective of this study was to understand payers' perspectives of unmet needs, value drivers and evidence requirements for Disperazol as well as exploring their willingness to pay for an adjunctive treatment. To explore this issue forty qualitative telephone interviews were conducted with payers and physicians across the EU5 and USA,

Results: The study identified a significant need for additional treatments to improve the long-term management of chronic *P.aeruginosa* infections in patients with CF. Treatments must focus on a reduction in exacerbations and subsequent hospitalisations, a reduction in the use of aminoglycosides, improvement of, or reduction in, lung function if they are to meet this clinical unmet need in a cost effective and clinically relevant manner.

Conclusion: The opportunity for a new drug to deliver desired clinical outcomes, whilst simultaneously reducing aminoglycoside use, is a pioneering approach to how new antibiotics can be valued in a proactive manner and will provide a valuable contribution to the antimicrobial stewardship agenda and ultimately the long-term health of people with CF.

A128

Targeting Antimicrobial Resistant *Staphylococcus aureus* In Chronic Wound Biofilms

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Abstract

Staphylococcus aureus is a pathogenic opportunist and one of the most frequently isolated organisms from various wound infections, associated with millions of deaths globally every year. *S. aureus* infection and persistence is mediated by the production of numerous virulence factors, and biofilm formation, which aids in host-immune evasion and development of resistance to systemic antibiotics. Phenotypic functional assays show that *S. aureus* strains exhibit high degree of genetic diversity. Understanding this intra-strain diversity is important for developing effective therapies that selectively inhibit *S. aureus* during chronic infections. Here, we have isolated clinical *S. aureus* strains from early-stage and end-stage chronic wounds and phenotypically screened for antimicrobial resistance (AMR) profiles and biofilm-forming capabilities. We reveal notable phenotypic diversity in biofilm formation and AMR profiles from isolated *S. aureus* strains, ranging from weak, intermediate and strong biofilm formers, and multidrug resistance to susceptible strains, respectively. Additionally, phenotypic profiles generated from late-stage chronic wound strains show increased AMR and biofilm formation compared to early-stage strains. Our data demonstrate the heterogeneity of *S. aureus* chronic wound isolates, which is being explored further via whole genome sequencing (PromethION). Metagenomic analysis will be carried out to reveal potential differences between early-stage and late-stage *S. aureus* isolates and conclusively correlate different virulence factors phenotypic profiles. Moreover, molecular analysis will be undertaken to uncover the nature of these biofilms, which will give insight to the structure of the extracellular matrix.

A129

No Smoke Without Fire: Microbial and AMR Risks of Cigarette Butts in London

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Abstract

Re-smoking of discarded cigarette butts - retrieving and reusing partially smoked cigarettes is a behavior reported among people experiencing homelessness, yet the associated infection and antimicrobial resistance (AMR) risks remain poorly understood. This study applies a metagenomics workflow to assess the public health risks posed by this practice in central London. Sampling sites were identified through community-led focus groups, which highlighted high-risk locations including train stations and a public house.

Discarded cigarette butts were aseptically collected and analysed to characterise microbial communities and resistance profiles. Initial findings reveal the presence of pathogenic bacteria, including members of the Enterobacteriaceae family, alongside oral, respiratory, and enteric taxa. Resistome analysis is ongoing, with early results suggesting the presence of mobile genetic elements and resistance-associated genes, indicating potential for horizontal gene transfer and environmental persistence.

The detection of viable pathogens and AMR markers on re-smoked cigarette butts represents a direct transmission risk, particularly for vulnerable individuals. These findings raise concerns about overlooked microbial hazards in urban public spaces.

By integrating community insight with high-resolution metagenomic analysis, this study highlights discarded cigarette butts as potential fomites and AMR vectors. Results will inform risk mitigation strategies, targeted public health messaging, and One Health surveillance frameworks focused on protecting vulnerable populations

A130

New Tricks for Old Bugs: Repurposing Phenothiazines as Antimicrobial Adjuvants to Treat Pseudomonas aeruginosa Infections in Cystic Fibrosis.

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Abstract

As antimicrobial resistance becomes an ever-worsening threat to the current reserve of available antibiotics, antimicrobial resistant Pseudomonas aeruginosa poses greater threats in individuals living with Cystic Fibrosis (CF), who are frequently under antibiotic treatments to combat chronic infections. Phenothiazines are a class of antipsychotic drugs that have shown antimicrobial activity against several bacteria and therefore are interesting candidates as antimicrobial adjuvants.To better understand the impact of thioridazine (TZ), chlorpromazine (CPZ) and promazine (PZ) on growth, virulence, and antibiotic susceptibility phenotypes, laboratory-based experiments were carried out in P.aeruginosa PAO1 and two clinical isolates obtained from CF patients.Growth kinetics were performed using the OmniLog platform; susceptibility to antibiotics and phenothiazines was determined using minimum inhibitory concentrations (MIC) by broth microdilution and disk diffusion assays.Following phenothiazine exposure, motility, biofilm production, pyocyanin quantification and cell morphology were assessed. Synergy assays investigated the effect of phenothiazines on enhancing the activity of the fluoroquinolone antibiotic ciprofloxacin. Changes were observed in growth kinetics of all strains when exposed to phenothiazines, with PZ having the greatest effect on doubling time and filamentation. General exposure to phenothiazines did not alter overall motility, biofilm formation, or antibiotic susceptibility profiles; however, one strain showed a shift in resistance when TZ and ciprofloxacin were combined. When strains were exposed to CPZ and PZ, there was increased pyocyanin production in all strains, whereas TZ did not induce this increase.This suggests phenothiazines have a pleiotropic effect on P. aeruginosa and that in clinical practice, they have potential to be used in combination with antibiotics.

A131

Rapid emergence of colistin resistance is driven by phenotypic and gene duplicated tolerant intermediates.

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Abstract

Colistin, a crucial last-line antibiotic, is often used to combat multidrug-resistant bacteria in humans and animals. However, clinical bacterial isolates frequently exhibit mixed populations where a predominant susceptible variant masks the existence of low-frequency and unstable colistin heteroresistant (HR) cells. However, the mechanism for the emergence of such variants and the establishment of permanent resistance to colistin is not well understood. Using a combination of a quantitative Resistance Emergence Assay (REA), single cell imaging, sequencing, and mathematical modeling, we demonstrate that bacteria tolerant to colistin can spontaneously emerge within a few generations even when exposed to concentrations that exceed the MIC. Further, we demonstrate that these low-frequency tolerant bacteria lead to the emergence of heteroresistance, contrary to prior postulates that attributed this to pre-existing strains. Early tolerant cells are dominated by altered transcriptional activity and gene duplications, albeit at the cost of fitness defects. Hence, these modifications are unstable and quickly lost in the absence of colistin. However, the occasional acquisition of site-specific point allows the bacteria to rid themselves of the gene duplications while fixing the resistance in the population. Colistin HR mutant strains and their tolerant precursors exhibit a characteristic modification of the lipid membrane environment that affects the binding and efficacy of colistin.

Overall, our study reveals that phenotypic tolerance, rapid gene duplication, and resistance fixation by site-specific mutations, rather than pre-existing resistant subpopulations, is a common pathway for the emergence of colistin resistance, highlighting the need to target transient bacterial intermediates to improve antibiotic treatment strategies.

A132

Revealing the secrets of the Mutuba tree: sustainable antimicrobial fabrics for healthcare and wellbeing.

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Abstract

Surgical site, soft tissue and wound infections are some of the most prominent causes of healthcare associated infections. The development of antimicrobial wound dressings is one method of reducing the transmission of bacterial pathogens in healthcare environments, whilst assisting the healing process and promoting localised antisepsis. Natural barkcloth fabrics derived from Mutuba tree species have been produced by communities in Southern Uganda for centuries in a highly skilful, fully sustainable and renewable process where bark is regenerated annually and Mutuba trees support ecosystems, agroforestry and wellbeing. This study identified that several barkcloth fabrics derived from different tree species possessed ideal properties associated with wound dressing technology, including antimicrobial (>7-log reduction) and antibiofilm (>4-log reduction) activity against multiple methicillin-resistant *Staphylococcus aureus* (MRSA) strains; excellent gaseous transmission; biocompatibility; mechanical protection and biodegradation. Scanning electron microscopy demonstrated physical morphological changes in the MRSA cell ultrastructure when bacteria were exposed to different barkcloth derivatives, which was also linked to the presence of natural surfactants. These properties, combined with the physical characteristics elicited by barkcloth suggest the fabrics are ideally suited for wound and other skin care applications. Ongoing studies into the chemical composition of different bark species, coupled with ethnobotanical and dermatological investigations further support the observed antimicrobial activity. This study is the first example where whole barkcloth products made by traditional methods have been employed as antimicrobial fabrics against MRSA and presents a major advance in the search for natural agents which could be deployed for healthcare and combatting antimicrobial resistance.

A133

Adaptation of Dual-MDR-Plasmid *E. coli* Under Differential Antibiotic Selection

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Abstract

Multidrug resistance (MDR) plasmids drive the spread of antimicrobial resistance (AMR) in *Escherichia coli*, which is an important cause of community and hospital-acquired infections. Pathogenic strains are commonly coinfected by multiple MDR plasmids, further expanding their breadth of AMR. Here, I test how *E. coli* coinfected with two clinically relevant resistance plasmids, pOXA-48 and pLL35, adapts to different antibiotic treatment regimens. Replicate populations were experimentally evolved for 28 days under ertapenem monotherapy, cefotaxime monotherapy, dual-drug treatment, alternating drug treatment, or drug-free conditions. Population densities and resistance phenotypes were tracked over time. For endpoint evolved clones, we quantified growth in antibiotic-free medium, resistance phenotypes, and genomic responses to selection. Whereas cefotaxime resistance was stably maintained in all lines, ertapenem resistance was maintained only under ertapenem treatment, but loss of ertapenem resistance was not caused by loss of pOXA-48. Regimens containing ertapenem selected for high level ertapenem resistance, but whereas this came at a cost to growth under monotherapy, this trade-off was avoided by clones that evolved under dual-drug selection. Drug-free selected lines, which evolved higher growth in the absence of antibiotics, gained parallel mutations in ribosomal proteins or global regulators. By contrast, ertapenem-selected lines gained parallel mutations in outer membrane porins or their regulators, commonly associated with reducing antibiotic influx. Dual-drug selected lines uniquely gained mutations in both outer membrane porins and ribosomal proteins, potentially suggesting how these lines evolved high level ertapenem resistance at low fitness cost. Overall, the results suggest that combination therapies may select for high-level resistance without fitness costs.

A134

AfuPmV-1 influences *Aspergillus fumigatus* drug susceptibility

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Abstract

Antifungal resistance is a critical yet under-reported component of antimicrobial resistance (AMR). *Aspergillus fumigatus*, the major cause of invasive pulmonary aspergillosis (IPA) in immunocompromised patients, exemplifies this threat. Rising resistance, driven by prolonged azole therapy and cross-resistance from agricultural fungicides, undermines the efficacy of first-line azoles, with mortality in resistant IPA cases exceeding 80%. An underexplored factor influencing antifungal susceptibility in *A. fumigatus* is the presence of mycoviruses. Mycoviruses are known to modulate fungal physiology, fitness, and drug responses. Therefore, this study investigated whether Aspergillus fumigatus polymycovirus (AfuPmV) 1 infection alters *A. fumigatus* susceptibility to antifungal agents, specifically echinocandins (β-1,3-glucan synthase inhibitors) and Nikkomycin Z (a chitin synthase inhibitor), and/or how its presence affects drug synergy. To this end, relative changes in antifungal susceptibility across virus-infected (VI), virus-cured (VC), and virus-reinfected (VR) isogenic lines were assessed using XTT-based metabolic assays. VI and VR isolates showed reduced baseline metabolic activity as compared to VC, consistent with known mycoviral impacts on fungal fitness. In single-drug assays, Nikkomycin Z inhibition was more pronounced in VI and VR as compared to VC. Caspofungin produced the strongest inhibition overall, with slightly higher tolerance observed for VC. Micafungin effects showed maximal differentiation based on AfuPmV-1 infection status, with VC being largely unaffected. Synergistic drug assays indicated that Nikkomycin Z potentiated echinocandin activity, particularly for Caspofungin in VI and VR. Although suboptimal concentration ranges precluded formal FICI analysis, these trends suggest that AfuPmV-1 modulates both monotherapy and synergistic antifungal responses in *A. fumigatus*, warranting further investigation.

A135

DISCOVERY OF NOVEL ANTIMICROBIAL PEPTIDES FROM NIGERIAN OIL-CONTAMINATED ENVIRONMENTS: TARGETING ESKAPE PATHOGENS AND BIOFILM.

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Abstract

Antimicrobial resistance (AMR) presents a serious global health challenge, highlighting the need for new antimicrobial agents. This study investigates hydrocarbon-contaminated soils in Nigeria as sources of antimicrobial peptides (AMPs), utilising microbial adaptations to oil pollution to fight AMR. Pollution alters microbial communities, enhancing bioactive compound production, including AMPs

Fifteen soil/sediment samples from five oil-affected sites were analysed through culture-based and metagenomic approaches. Culture-based isolation yielded 56 strains, with 14 active against ESKAPE pathogens. Metagenomics involved DNA extraction, Illumina sequencing, contig assembly, and ORF prediction, identifying 73 (AMPA) and 95 (Macrel) AMPs, refined to 12 and 28 candidates via APD3 criteria. Additionally, 38 Metagenome-Assembled Genomes (MAGs) were generated, with 10,543 (AMPA) and 111,064 (Macrel) AMPs streamlined to 358 and 3, respectively, for synthesis.

Ten candidate AMPs were synthesised; three exhibited activity. ZB8 displayed potent MICs of 16 µg/mL against MRSA 33592 and S. *aureus*ATCC 43300, 32 µg/mL against E. *faecium*DSM 25390 and E. *coli*ATCC 10488, and 64–128 µg/mL against K. *pneumoniae*NCTC 204 and A. *baumannii*NCTC 13304, with MBCs of 128–256 µg/mL. Biofilm assays confirmed MBIC/MBEC.

These results confirm that oil-contaminated Nigerian sites are sources of unique AMPs to combat AMR. Future efforts include expanded synthesis, optimisation, and in vitro validation with clinical isolates and toxicity profiling.

A136

Development of a Rapid PCR-Based Point-of-Care Diagnostic Kit for Detection of Antimicrobial Resistance Genes

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Abstract

Antimicrobial resistance (AMR) continues to threaten effective infection management, particularly in low-resource healthcare settings where conventional antimicrobial susceptibility testing (AST) requires 24–72 hours. This delay promotes empirical use of broad-spectrum antibiotics and accelerates resistance. The present study describes the design and validation of a rapid, PCR-based molecular diagnostic kit for point-of-care detection of AMR genes directly from clinical samples. The prototype employs multiplex real-time PCR with custom primers and TaqMan probes targeting blaCTX-M-15 and aac(6')-Ib-cr genes, responsible for β-lactam and fluoroquinolone/aminoglycoside resistance, respectively. DNA was extracted using the HiGeno MB rapid kit, ensuring high-quality nucleic acid recovery within 45 minutes. The complete workflow—from sample to result—was optimized to deliver interpretable outputs in under 60 minutes using portable qPCR platforms. Comparative analysis with conventional AST showed 100 % sensitivity and 87.5 % specificity, confirming diagnostic reliability. The portable system integrates simplified DNA extraction, closed-cartridge amplification, and fluorescence-based detection, offering a compact, field-ready solution. Ongoing optimization addresses reagent stability, power constraints, and cost through lyophilized reagents, solar-powered thermocyclers, and modular design. This innovation demonstrates how rapid molecular diagnostics can revolutionize AMR surveillance and antibiotic stewardship by enabling accurate, timely detection at the point of care—ultimately reducing unnecessary antibiotic use and improving patient outcomes in both urban and rural healthcare settings.

A137

Diagnostic-guided antimicrobial therapy: Transforming early antibiotic decision-making to combat AMR

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Abstract

Antimicrobial resistance (AMR) is a rapidly escalating global health threat, driven in part by the widespread practice of initiating empirical antibiotics before pathogen identification. This contributes to inappropriate prescribing, increased costs, prolonged recovery, therapy failures, and the emergence of resistant organisms. Integrating rapid diagnostic tools—such as procalcitonin biomarker testing and accelerated culture methods—offers a promising strategy to optimise early antibiotic decisions and strengthen antimicrobial stewardship. This study evaluates the clinical and economic impact of diagnostic-guided therapy among adult ICU patients in a 750-bed teaching hospital in Vadodara, Gujarat. Over 12 months, two cohorts will be compared: Group A receiving standard empirical therapy and Group B managed using rapid diagnostic and biomarker-based guidance aligned with stewardship principles. Eligible participants (≥18 years) include patients with suspected respiratory, urinary, bloodstream or intra-abdominal infections and an anticipated ICU stay of at least 48 hours. Data collection will capture demographics, APACHE-II/SOFA scores, infection characteristics, laboratory parameters, antibiotic appropriateness and duration, ICU and hospital length of stay, mortality, and treatment-related costs. Diagnostic-guided therapy is expected to improve early appropriateness of antibiotics, enhance clinical outcomes, shorten recovery time, reduce complications, and decrease hospital costs by limiting unnecessary broad-spectrum antibiotic use. Findings will provide robust evidence on the effectiveness, feasibility, and cost-efficiency of rapid diagnostics in guiding antibiotic decision-making, supporting policy development and targeted stewardship interventions to curb AMR in resource-limited healthcare settings.

Session : Microbial Physiology, Metabolism and Molecular Biology Forum

A138

The glucose uptake inhibitor SgrS is induced by D-serine yet does not contribute to growth arrest in enterohaemorrhagic E. coli

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Abstract

Host-derived metabolites regulate bacterial physiology and contribute to infection dynamics. D-serine, abundant in extraintestinal environments but scarce in the gut, serves as a niche-specific signal influencing *E. coli* pathotype adaptation and virulence. In enterohaemorrhagic *E. coli* (EHEC), a major foodborne pathogen, D-serine exposure impairs growth, but the mechanisms underlying this toxicity remain unclear.

Transcriptome datasets were analysed to identify differentially expressed genes in response to D-serine. Growth inhibition was evaluated across minimal media with diverse carbon sources and rescue assays with specific metabolite supplementation were performed. Deletion of *sgrS* was used to assess its role in D-serine toxicity. Further testing with the SgrS mutant was used to monitor activity of the known D-serine targets, LEE1 and RecA.

The small RNA SgrS, canonically linked to glucose-phosphate stress, was identified as the most strongly upregulated transcript. However, growth inhibition remained unaffected across diverse carbon sources, including those independent of SgrS-regulated transport. Deletion of *sgrS* did not restore growth nor did supplementation with pyruvate or pantothenate, while L-serine provided partial rescue, implicating disruption of fundamental metabolic processes. Reporter assays confirmed D-serine-induced repression of LEE1 and induced SOS response activity occurs independently of SgrS.

D-serine elicits a pathotype-specific response in EHEC that disrupts central metabolism and stress regulation. While not directly responsible for growth inhibition, the strong induction of SgrS reflects indirect activation of stress pathways and reveals a broader transcriptomic reprogramming than previously recognised. These findings demonstrate how host-derived metabolites can trigger diverse regulatory networks and expose unique vulnerabilities in pathogenic *E. coli*.

A139

The dynamic physiological states of the bacterial transcription terminator, Rho, regulate its *in vivo* functions.

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Abstract

Bacterial transcription terminator Rho is an RNA helicase that regulates physiological processes. Its *in vivo* regulation might be spatial localization-dependent. Here, we investigate the physiological state(s) of *E. coli* mCherry-Rho using SIM-Microscopy. In dividing cells, Rho resides on the inner membrane as puncta, with a few molecules present in the cytoplasm near DNA. A significant number of Rho molecules are seen on the DNA upon crosslinking, transiently being present near the transcription process. At slow growth rates, it forms an annular ring on the inner membrane. It is delocalized in the cytoplasm around the DNA during the stationary phase, and when Rho-helicase function is impaired, suggesting that membrane anchoring is crucial for unwinding RNA. The presence of Rho near the DNA is required to stop unwanted transcription at the stationary phase. Rho forms an inactivated concentrated sphere reversibly at one of the poles when (p)ppGpp level rises, indicating its inactivation during the stringent response. Rho shares the space with ribosomes on the inner membrane, implying that it remains anchored to the RNA part of the polysomes. NusG stays “sandwiched” between the ribosome and the peripheral parts of the chromosome. However, it colocalizes with Rho when transcription is inhibited. We concluded that Rho is regulated by “compartmentalization” at different locations *in vivo* depending on the metabolic needs. We surmise that the RNA-helicase function of Rho is essential to stay anchored to the membrane-bound mRNA, enabling it to unwind RNA secondary structures before being chaperoned to the RNA degradosome complexes.

A140

Further understanding the role of 7α-dehydroxylation in resistance against *Clostridioides difficile*

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Abstract

A healthy microbiome provides resistance against *Clostridioides difficile* infections (CDI) through various mechanisms, including the metabolism of primary bile acids. Antibiotic use is a major risk factor for CDI due to subsequent disruption to the gut microbiome. An example of this is the loss of individual species which perform protective mechanisms. For example, certain species of *Clostridia* possess the enzymatic capability to metabolise primary bile acids, which are known to initiate *C.difficile* spore germination, via the process of 7α-dehydroxylation. They are converted into secondary bile acids, which are demonstrated to inhibit spore germination and outgrowth, vegetative growth and toxin activity of *C.difficile.* Thus, it is hypothesised that the loss of primary bile acid metabolism contributes to the development of CDI. Furthermore, it has been demonstrated that healthy individuals have significantly higher levels of secondary bile acids and significantly lower levels of primary bile acids than CDI patients.

This study aims to further characterise 7α-dehydroxylation of primary bile acids, and its role in CDI resistance, alongside developing probiotic strains which modulate bile acid metabolism, offering a potential preventative measure for CDI. Through Allele Coupled Exchange, the bile acid-induced operon was inserted into the chromosome of *Clostridium butyricum*, a species with a long history of safe use as a probiotic and treatment for antimicrobial-associated diarrhoea. Alongside this, there has been evidence of *C.butyricum's* intrinsic capacity to enhance resistance against CDI. This genetically engineered strain will be used to assess the extent of protection the metabolism of primary bile acids provides against CDI.

A141

Alarmone-mediated regulation of ribosomes – contributors to high-level antibiotic resistance in MRSA?

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Abstract

The stringent response is conserved across bacterial organisms as a means of survival in response to nutrient deprivation. The response enhances the production of secondary signalling molecules referred to as alarmones, specifically guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp). These alarmones regulate crucial metabolic pathways in the cell, reducing DNA replication, nucleotide synthesis, transcription as well as ribosomal maturation and function. We have previously observed that upon induction of the stringent response in *Staphylococcus aureus*, the formation of 70S ribosomes is inhibited by obstruction of the interactions between ribosomal particles and a family of enzymes termed the RA-GTPases (ribosome associated GTPases); which are essential for maturation and regeneration of ribosomes. (p)ppGpp are also responsible to reduced rRNA transcription upon stringent response activation. More recently, we have also observed that alarmones levels are raised in and contribute to homogenous high-level b-lactam resistant *S. aureus* strains. Here, we explore the correlation between the stringent response, ribosomal levels and structure with high-level antibiotic resistance in *S. aureus.*

A142

The effect of black carbon on *Moraxella catarrhalis*

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Abstract

The WHO categorises air pollution among today's major global risks to human health. Fine particulate matter (PM2.5), which black carbon is a major component of, has been shown to have a detrimental effect on human respiratory health. This is partly due to direct impacts of pollutants on the respiratory tract. However, one aspect of this association that is consistently overlooked, is the effect air pollutants can have on bacterial pathogens, namely increasing their pathogenicity and ability to colonise.

Moraxella catarrhalis, a common causal agent of otitis media and COPD exacerbations, is among those species hypothesised to be affected by air pollution, due to trends seen in both epidemiological and microbiome data. Our current work shows that black carbon directly affects *M. catarrhalis'* tolerance to antibiotic exposure and ability to form stable biofilms. Furthermore, through analysis of transcriptional and genomic data, our work explores the mechanisms underlying these phenotypes, including the identification of potential novel regulators.

A143

Exploring strain-specific D-glutamate sensitivity of pathogenic *E. coli* using lab-based evolution

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Abstract

Unlike L-amino acids, D-amino acids are non-proteinogenic constituents in bacteria. Instead, these chiral enantiomers of L-amino acids have been shown to act as important signalling molecules, and are used to crosslink the cell wall. D-glutamate is one of these cell wall constituents, and through this role adds stability to the cell envelope.

Despite being an essential cell wall component, we observed that exposure to exogenous D-glutamate imparted a significant inhibitory effect on the growth of specific *E. coli* strains in select media. Specifically, growth of BW25113, NRG857c, and TUV93-0 was inhibited in LB+D-glutamate. Minimal media+D-glutamate inhibited growth of CFT073, BE2348, MG1655, NRG857c, TUV93-0 and UTI89. And MEM+D-glutamate inhibited growth of CFT073, TUV93-0, LF82 and UTI89. However, growth of LF82 and NRG857c was increased in LB+D-glutamate, minimal media+D-glutamate and MEM+D-glutamate respectively. Based on these observations, it appears that specific *E. coli* strains can not only be resistant to 'toxic' levels of D-glutamate but also thrive in its presence.

To investigate the mechanism of D-glutamate toxicity, we performed laboratory-based evolution experiments using the D-glutamate-sensitive strain TUV93-0. This strain was repeatedly passaged for 20 days in an inhibitory concentration of D-glutamate in minimal media. Every day throughout the 20 days, we isolated several individual strains which displayed partial or complete tolerance to typically toxic concentrations of D-glutamate. By analysing the genomes of these gathered isolates we aim to elucidate the mechanistic basis of D-glutamate resistance development, thereby establishing the underlying cytotoxic effect of D-glutamate in *E. coli*.

A144

The implication of evolutionary lineage variation found in *Proteus mirabilis* from seeds of *Pentaclethra macrophylla.*

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Abstract

The seeds from the plant *Pentaclethra macrophylla,* also known as *Ugba,* are widely consumed in South-Eastern Nigeria and play a huge socio-economic role in the lives of the people. The consumption of its fermented seeds has become a concern because fatalities after its consumption have been reported in many different local communities. The source of the food poisoning was not investigated or identified, and could be due to microbial proliferation before or after harvest. Post-harvest contamination by microorganisms may be due to poor handling during processing, storage, and sales. Recently, several strains of *Proteus mirabilis* were found in the seeds after molecular evolutionary analysis in our study. Seed samples from 10 geographical locations were sourced from trade and subjected to Illumina whole-genome sequencing. The strains were separated into different clades or monophyletic groups, which indicate distinct evolutionary lineages. *P. mirabilis* mode of existence in humans is known to be both intra- and extracellular, and in other bacteria like *Listeria monocytogenes*, certain lineages are more pathogenic than others. Here, our findings reveal that the factors driving the evolution of *P. mirabilis* strains in the seeds of *Pentaclethra macrophylla* analysed were driven by genetic drift or niche adaptations in addition to selective pressures like geographic location. The study shows the need for further research to identify mutation shifts and the resilient nature of the isolates. Tracking the genetic diversity of bacterial transmissions in Nigeria will help establish early warning systems for control of diseases that have public health significance.

A145

Microbial and Metabolic Mediators of Host–Microbe Dialogue in Early Life

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Abstract

Gut-resident bacteria produce a diverse array of metabolites, either directly synthesized or derived from dietary and host substrates. These metabolites, among them lipid mediators, can signal to influence gut health, maintain immune and metabolic homeostasis, and enable communication between the gut and distant organs. Critically, these biochemical interactions may shift with microbial dynamics across life stages, forming unique species and strain level metabolic fingerprints. The common ground in strain and species shifts lies in their functions—the metabolites they produce or transform. Among early colonisers, Bacteroideales (notably *Bacteroides*) mega-lipid producers and Proteobacteria (including *E. coli*) oxygen depleters, are abundant but remain under characterised in terms of their metabolite contributions to the host–microbe interactions. We aimed to understand the metabolite contributions of these bacteria, once isolated from the infant gut.

Why is this important? These first gut colonizers are understudied, they represent an untapped potential probiotic resource, they are not yet extensively and experimentally characterized, as individuals, for any unique features that may impact colonization, gut development, and immune system training among other functions, diet may alter their metabolite landscape. Studying isolated individual strains and their genetics may reveal functional contributions that influence early life development, for longer term health.

A146

Identification of a water filled hemichannel within a sensor kinase governing antibiotic influx into Gram-negative cells

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Abstract

In an age where multidrug resistance is becoming more prevalent, there is need for novel drug targets. Gram-negative bacteria contain porins in the outer membrane, and control influx of antibiotics. The alteration in the expression of porins is one of the methods contributing to multidrug resistance. In Escherichia coli, the well understood two-component system (TCS) EnvZ/OmpR regulates outer membrane porins OmpC and OmpF. EnvZ exhibits cytoplasmic osmosensing and periplasmic allosteric interaction with MzrA to change the ratio of EnvZ kinase and phosphatase activity. To monitor the position and dynamics of helices in signal transduction, we performed in vivo disulphide crosslinking within the transmembrane domain (TMD) between EnvZ and MzrA across ~250 Cys substituted pairings. We found Cys crosslinking between EnvZ and MzrA at the cytoplasmic and periplasmic ends of the TMD, indicating these residues face towards one other in a close enough proximity for crosslinking to form. We also analysed in vivo signal output across 15 Cys substitutions in the cytoplasmic domain of EnvZ and the increase in signal output in the N-terminal towards the TM region suggests the presence of a water filled hemichannel. Furthermore, we propose that these water filled hemichannels are a conserved motif in sensor kinases and promote transmembrane communication.

A147

Cystic fibrosis airway-associated pathogens display strong signatures of adaptation in a polymicrobial lung infection model.

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Abstract

People with cystic fibrosis (pwCF) frequently suffer from chronic airway infections. Complications from these infections remain a leading cause of morbidity and mortality in pwCF. These airway infections are often polymicrobial, with multiple species of Gram-negative and Gram-positive bacteria, and fungi co-colonising the airway niche. Key species observed at high frequency in the CF airways, or associated with worsened pulmonary health, include the WHO-designated 'high priority' pathogen *Pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus* (Gram-positive), *Stenotrophomonas maltophilia* (Gram-negative), and *Candida albicans* (fungus). Although often clonally-derived, the populations of each species are rarely homogenous, with certain genetic mutations observed at higher frequency than expected under genetic drift. This indicates that these mutations are 'pathoadaptive' – that they increase fitness within this highly-competitive environment. However, we still have little idea as to why this is, and our knowledge of the impact of these mutants on the ecology of the microbial community remains limited. In this study, we are using genomic analyses (and later, gene expression profiling) to explore the mutational dynamics of these key CF-associated species, as well as investigating how the acquisition of key mutations affects inter-species interactions and the response to antibiotics. This is being done using an *in vitro* polymicrobial culture setup which captures the inter-species dynamics observed in the CF airways. An improved understanding of the factors that drive these mutants to arise, and their influence on other co-habiting microbes within the CF airways, is critical for the continued development of more effective treatments to manage airway infections.

A148

Lipoxin A4 Modulates *Staphylococcus aureus* Growth and Stress Tolerance Differentially in Methicillin-Resistant Strains

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Abstract

Chronic wound infections are fundamentally characterized by dysregulated healing and exacerbated by microbial biofilms, thus making the modification of the host's inflammatory response via endogenous Specialized Pro-Resolving Mediators (SPMs) like Lipoxin A4 (LXA4) an attractive therapeutic strategy.

While the anti-inflammatory and pro-healing role of endogenous Lipoxin A4 (LXA4) in wound contexts is established, the nature of its direct interaction with key bacterial pathogens like *Staphylococcus aureus* remains a critical area of investigation. In this study, we investigated the direct effect of LXA4 on biofilm formation and planktonic growth in various *Staphylococcus aureus* strains, including Methicillin-sensitive (MSSA) and Methicillin-Resistant (MRSA). Biofilm biomass was quantified using crystal violet staining, and planktonic cell growth was monitored over time in the presence of varying LXA4 concentrations.

Our experiments revealed that LXA4 demonstrated minimal to no anti-biofilm activity against either MSSA or MRSA. However, analysis of planktonic growth revealed a dose-dependent interaction: LXA4 exhibited significant protective and proliferative effects on MRSA growth, but not on MSSA growth, especially during the stationary phase. This protective effect was observed against the inhibitory influence of the ethanol vehicle used for LXA4 delivery, suggesting a unique MRSA-specific mechanism of stress tolerance mediated by the lipoxin. This finding suggests that LXA4, while beneficial to the host, may inadvertently act as a pro-survival signal for MRSA.

Future work will utilise *in vivo* human epithelial skin model to assess the effects of LXA4 in a complex host-pathogen system, specifically evaluating its impact on epithelial cell repair and bacterial virulence factors.

A149

Pollution and Prokaryotes: Exploring the Impact of Particulate Air Pollution on Methicillin-Resistant *Staphylococcus aureus*

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Abstract

According to the World Health Organisation, air pollution from fine particular matter ($PM_{2.5}$) caused 4.2 million premature deaths worldwide in 2019. $PM_{2.5}$ is released as a result of industrial and vehicle emissions, as well as brake and tyre wear. It has been shown that exposure to PM, of which black carbon (BC) is a major component, not only has harmful effects on human respiratory systems, but also alters the behaviour of the bacteria that colonise us. In our publications we have shown that Methicillin-Resistant *Staphylococcus aureus* has an increased ability to adhere to and invade respiratory epithelial cells after exposure to and co-exposure with BC, as well as *in vivo* an increased colonisation of the murine respiratory tract, and alterations to biofilm antibiotic tolerance. We have shown BC directly influences the differential expression of a range of *S. aureus* genes, including those associated with toxin production and immune evasion (including *hla*, *chp*, and *psmα* and *β*). Our current work involves exploring the physical and chemical changes to the cell, in addition to understanding the mechanisms behind this adaptive response. We are also exploring how these changes impact the susceptibility to antibiotics and alter host-pathogen interactions, so we can begin to understand how rising levels of air pollution across the globe will alter *Staphylococcal* infections in urban populations.

A150

The stringent response is required for *Staphylococcus aureus* survival in the oropharynx.

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Abstract

Staphylococcus aureus is an opportunistic pathogen and a common coloniser of both the nares and oropharynx, colonising between 15-30% of the population at any given time. Antimicrobial-resistant and antimicrobial-tolerant bacterial infections have been increasing in prevalence in recent years, pushing the need for a greater understanding of bacterial colonisation and survival within the human host. *S. aureus* possesses the ability to survive for prolonged periods in human saliva, a relatively nutrient-deplete bodily fluid, a trait that may enable its colonisation of and subsequent survival in the oropharynx. One bacterial stress response known to be important in nutrient-depleted conditions is the stringent response. This response is mediated by a set of small, phosphorylated nucleotide messenger molecules, guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp). Following the activation of the stringent response, the intracellular concentration of (p)ppGpp rises, enabling the shut-down of short-term growth and promoting bacterial survival. Our preliminary experiments have demonstrated that (p)ppGpp production is essential for this prolonged survival in saliva. Following this, we will now dissect why, as well as establish the importance of the stringent response in the ability of *S. aureus* to colonise the oropharynx. A greater understanding of the role of the stringent response in intracellular staphylococcal infections could aid in targeted treatment of recurrent tonsilitis, thereby reducing the need for young children and adolescents to undergo tonsillectomies, and could provide vital insight into how *S. aureus* causes recurrent, antimicrobial-tolerant infections in other bodily sites as well.

A151

Investigating maturation and egress from host cells in the obligate intracellular bacterium *Orientia tsutsugamushi*

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Abstract

Orientia tsutsugamushi (Ot) is a mite-borne, obligate intracellular bacterium and a causative agent of the human disease scrub typhus. Ot targets a wide range of tissues, leading to CNS complications, multi-organ failure and mortality in severe cases. Previous investigation has focused on the initial stages of the Ot lifecycle, including entry into cells, intracellular motility and bacterial replication in the cytosol. However, it is not known how the bacterium exits host cells, nor how this process is induced.

To address this, I have developed a microscopy-based assay to quantify bacterial maturation and egress. It is not known whether bacterial exit is purely time-dependent, or whether bacterial copy number impacts maturation, implying that bacteria may sense each other or conditions in the host cell. Using this assay, I show that initial bacterial number impacts maturation, with high MOI infections reaching a maturation state earlier. To address the question of whether bacteria need to be metabolically active to mature, I combine this assay with antibiotic treatments. I show that Ot require active bacterial protein synthesis to move to the cell periphery, but not to egress.

It is unclear whether Ot maturation is triggered by intracellular conditions or by a maturation process intrinsic to bacteria. To untangle this complexity, I use a panel of chemical inhibitors to disrupt specific host and bacterial pathways. Preliminary results suggest that maturation may be triggered early by changes in nutrient availability or actin polymerisation; further work will focus on understanding how these changes induce Ot maturation.

A152

Investigating population heterogeneity of *Orientia tsutsugamushi* using spatial transcriptomics

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Abstract

Orientia tsutsugamushi is an obligate intracellular bacterium and causative agent of scrub typhus, a mite-borne disease that represents a major cause of non-malarial febrile illness across South and Southeast Asia. The bacterium undergoes a complex intracellular life cycle, transitioning from replicating in a perinuclear microcolony within host cells to an extracellular form that buds from the cell surface in a virus-like manner. Bulk RNA sequencing has revealed distinct transcriptional profiles between these intracellular and extracellular states, while RNAscope analyses indicate substantial heterogeneity in bacterial gene expression between individual bacterial cells within one infected host cell. However, a global, single-bacterium–level characterisation of transcriptional heterogeneity during the intracellular life cycle has not yet been performed. To address this, we apply spatial transcriptomics to map bacterial gene expression at subcellular resolution. Using a custom 200-gene panel, we will perform 10x Genomics Xenium spatial transcriptomics to profile *O. tsutsugamushi* populations within infected mammalian cells. This approach will enable the identification of transcriptionally distinct bacterial subpopulations and provide new insights into the spatial organisation of bacterial states within host cells. To our knowledge, this represents the first application of spatial transcriptomics to study gene expression in an obligate intracellular bacterium.

A153

Microbial functional interactions in carbonate chimney structures of Red Sea hydrocarbon seeps

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Abstract

In the Red Sea, the seafloors surrounding sulfidic brine pools can be characterised by distinctive chimney-like structures which represent biogeochemical and ecological hotspots in an otherwise oligotrophic environment. Such remarkable ecosystems are driven by chemosynthetic microbial communities adapted to exploit the hydrocarbon-rich, reduced fluxes seeping from the seafloor while contributing to the chimney formation.

This study focuses on an active chimney field discovered in the southern Red Sea, offering a rare window to investigate such fascinating yet enigmatic systems. By investigating the composition and functions of the microbial communities inhabiting different portions of the chimney structures, the research aims to elucidate: (i) the geomicrobiological processes driving the chimney formation; (ii) the metabolic interactions mediating biogeochemical fluxes critical to chimney ecosystem functioning; (iii) the symbiotic partnerships facilitating the adaptation of macroorganisms inhabiting the chimney field.

Integrated metagenomic and metatranscriptomic analysis identified anaerobic methanotrophic (ANME) archaea and sulphate-reducing bacteria (SRB) as abundant and metabolically active within the inner anaerobic chimney core, oxidising alkanes as their primary carbon and energy source. Metabolites from alkane degradation likely drive mineral precipitation processes and fuel other microbial taxa. On the outer surface, exposed to oxic seawater, microbial mats dominated by *Beggiatoaceae* oxidise hydrogen sulphide derived from chimney fluids or microbial sulphate reduction. Furthermore, the identification of *Sulfurovum* species associated with the gills of crabs inhabiting the chimney surrounding suggests a symbiotic relationship, in which these bacteria may contribute to detoxifying hydrogen sulphide, facilitating host adaptation to seafloor conditions.

A154

Production of Mycophenolic Acid and Roquefortine C by Penicillium roqueforti Isolates from Turkish Blue Cheeses

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Abstract

Konya Kuflu Tulum, Erzurum Kuflu Civil, and Divle Cave Tulum are among the most famous Turkish blue cheeses, produced through spontaneous fermentation. The distinctive blue-green color of these cheeses is primarily attributed to the filamentous fungus *Penicillium roqueforti*. This species also produces the main mycotoxins, mycophenolic acid (MPA) and roquefortine C (ROQC). This study aims to determine the production of mycotoxins (MPA and ROQC) by 20 different *P. roqueforti* isolates and to investigate their potential in cheese production. Mycotoxin levels of the isolates were analyzed on yeast extract sucrose agar (YESA) using high-performance liquid chromatography (HPLC). The concentrations of MPA and ROQC in the samples were quantified as <745.4 µg/kg and <963.5 µg/kg, respectively. Model cheese production was carried out using three isolates with distinct technological properties, along with CECT 2905 neotype strain. Following a ripening period of six weeks at 10 °C, some physicochemical and microbiological properties of the cheeses were evaluated, and the levels of MPA and ROQC were determined. The amounts of MPA and ROQC in the cheeses were found to be <29.5 µg/kg and <0.8 µg/kg, respectively. The results show considerable variation in production levels among isolates on YES agar, and these levels are significantly reduced in cheese compared to artificial media.

A155

Unravelling biofilm complexity in Gram-positive pathogens: *Enterococcus faecalis* and *Staphylococcus aureus* in focus

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Abstract

Biofilms are a key method of persistence and resistance for bacteria. Biofilms are generated by communities of bacteria and comprise a self-produced matrix of polysaccharides, proteins and DNA. They allow bacteria to adhere to biotic and abiotic surfaces, providing a stable environment for growth and protection against external stressors like antibiotics. This research will focus on imaging *Enterococcus faecalis* and *Staphylococcus aureus* biofilms using mesoscopy: an advanced microscopy technique that couples sub-cellular resolution imaging with a millimeter-scale field of view. Prior work revealed previously unknown architectural complexity within colony biofilms of the Gram-negative bacilli *Escherichia coli* and *Pseudomonas aeruginosa* imaged using mesoscopy, including the presence of a network of water and nutrient channels (DOI: 10.1038/s41396-020-0700-9). This study aims to determine if analogous structures exist within colony biofilms of Gram-positive bacteria, and to assess the impact of cellular arrangement on biofilm architecture. We will use widefield confocal mesoscopy to image colony biofilms of fluorescently labelled *E. faecalis* and *S. aureus*, to determine if analogous structures are present. In addition, since staphylococci grow as clusters of cells while enterococci exist as chains of diplococci, we will interrogate whether cell arrangement impacts the spatial distribution of the biofilm. We anticipate that mesoscale imaging of these biofilms will offer novel insights into microbial community structure and nutrient acquisition in these clinically important species.

A156

Mucin on the Menu: Degradation and Cross-Feeding of Mucin between *Staphylococcus aureus* and the Nasal Microbiome

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Abstract

Staphylococcus aureus is a leading cause of healthcare-associated mortality but also a human commensal, with 30-50% of adults colonised at some point. The primary niche of *S. aureus* is the nose, and nasal carriage is a prominent risk factor for infection. For stable colonisation, bacteria must acquire nutrients within their niche. In the nose, mucus which is rich in complex mucin glycoproteins, is a prominent nutrient source. Bacteria can harbour glycosyl hydrolase enzymes which cleave specific glycosidic bonds on the mucin carbohydrate branches. However, data from the CAZy database show *S. aureus* lacks known mucin-specific hydrolases, raising questions about how it accesses this nutrient source.

Here, we investigate the ability of genetically diverse clinical *S. aureus* strains to hydrolyse and grow on mucin in comparison to other nasal microbiome species. Our data show that *S. aureus* grows poorly when mucin is the sole carbon source and zone of clearance assays reveal that *S. aureus* cannot hydrolyse mucin. In contrast, other nasal microbiome species can hydrolyse mucin, and grow well, if not better, in minimal media supplemented with mucin compared to glucose. In other mucosal niches, such as the gut, mutualistic breakdown and cross-feeding of mucin degradation products occur. Our data suggests that *S. aureus* must depend on co-colonisers to utilise mucin. We will present data on these interspecies relationships, highlighting potential cross-feeding networks between nasal co-colonisers and showing the ability of *S. aureus* to utilise metabolic by-products of mucin degradation from other nasal microbiome species.

A157

Resolving the *Treponema pallidum*-host interaction using dual RNA-seq

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Abstract

Syphilis vaccine development is hampered by the poor understanding of *Treponema pallidum* (TP) biology and pathogenesis. TP was long considered non-culturable until a co-culture system with rabbit epithelial cells (Sf1Ep) was developed in recent years. While this system has enabled several discoveries, it remains technically demanding and cannot readily culture samples directly from patients without prior rabbit passage, making it unsuitable for clinical or LMIC settings. TP's dependence on host-derived nutrients, due to its small genome and limited biosynthetic capacity, further complicates axenic culture development.

Our project aims to obtain the information needed to optimize a cell-free culture that enables TP growth from clinical samples. To achieve this, we are using dual RNA-seq to examine transcriptional responses of both TP and Sf1Ep cells during infection. We sequenced the Sf1Ep genome using PacBio HiFi reads and annotated it in collaboration with the EBI. This annotated genome was used as a reference to map reads from 60 directional RNA-seq libraries derived from cells infected with different TP strains at various time points, along with corresponding non-infected controls. Our analysis focuses on the transcriptional responses of both TP and Sf1Ep cells, with the aim of identifying TP genes involved in host interaction as well as host metabolic pathways modulated during bacterial attachment. While we have identified multiple differentially expressed genes, future work will integrate these dual transcriptomic data with untargeted metabolomics to model TP–host metabolic exchanges and identify the essential nutrients required to support axenic TP growth.

A159

Stress and Quorum Sensing-Dependent Regulation of a Putative Lipoprotein in *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is a highly adaptable pathogen capable of causing a wide spectrum of infections, ranging from mild skin lesions to life-threatening disease. Its adaptability and pathogenic success are largely driven by complex regulatory networks that finely control virulence gene expression in response to environmental changes. To survive and persist across diverse host environments, *S. aureus* must continuously modulate the expression of its virulence factors. Among these, surface-associated bacterial lipoproteins (Lpp) play key roles in immune evasion, host interaction, and nutrient acquisition. Despite their importance, many *S. aureus* Lpp remain poorly characterised.

In this study, we investigated the regulation of a putative lipoprotein gene in *S. aureus*. Using an *S. aureus* transposon mutant library and a transcriptional reporter system, we identified mutants showing reduced Lpp expression, particularly those disrupted in key regulatory pathways associated with environmental stress responses, quorum sensing, and metabolism.

Our findings suggest that expression of this Lpp is controlled by stress and quorum-sensing pathways, underscoring its potential role in virulence regulation and environmental adaptation. These results highlight the interconnectedness of metabolic, stress, and signalling networks in *S. aureus*, and lays the groundwork for future investigations into the functional relevance of specific Lpp in bacterial survival under host-induced or environmental stress.

A160

Transcriptomic analysis of colony biofilm formation by a *dnaK* deficient *C. difficile* 630 mutant

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Abstract

The opportunistic enteric pathogen *Clostridioides difficile* causes significant healthcare problems due to the recurrent nature of infections attributed to the ability of *C. difficile* to form biofilms and spores within the gut. Disruption of the key chaperone gene, *dnaK*, by ClosTron mutagenesis yielded a DnaK deficient strain (630Δ*erm:dnaK*) which exhibited 50 % greater cell elongation, increased cell hydrophobicity, and increased biofilm production).

We used a colony biofilm model to investigate gene expression in 24h *C. difficile* biofilms and in planktonically grown cells comparing mutant strain (630Δ*erm:dnaK*), parent strain (630Δ*erm*) and wild-type (630). RNA sequencing revealed major transcriptional differences caused by disruption of the *dnaK* gene in 630Δ*erm:dnaK*. Gene set enrichment analysis identified increased expression of peptidoglycan biosynthesis in 630Δ*erm:dnaK* biofilm formation that was not observed in the other strains. However, all strains showed a similar increased expression of the sporulation pathway and a decrease in flagellar assembly when comparing 24h biofilms to planktonically cultured cells. Other indications of cell wall restructuring in 630Δ*erm:dnaK* included over representation of vancomycin resistance genes and glycerophospholipid metabolism KEGG pathways. Further biological processes such as cell division, cell wall organization, regulation of cell shape and peptidoglycan biosynthetic process were also overrepresented.

The novel transcriptional changes in cell wall related processes and pathways may explain phenotypical changes in cell morphology including cell elongation, increased hydrophobicity and recalcitrance to antibiotics previously reported by our group.

A161

Exploring the *C. difficile* Stringent Response

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Abstract

Clostridioides difficile infection (CDI) is the worldwide leading cause of infective nosocomial diarrhoea, constituting a significant global burden due to the consistently rising morbidity and mortality rates associated with the condition. *C. difficile* is rapidly becoming resistant to current treatments, highlighting the need for alternative therapies.

The stringent response is a highly conserved bacterial signalling pathway that responds to a wide range of stimuli, including but not limited to nutrient starvation, oxygen concentration, and pH change. The response is characterised by rapid synthesis of signalling nucleotides collectively referred to as (pp)pGpp, and is found in most bacteria. The outcome of stringent response activation differs between species but broadly includes an increase in branched chain amino acid synthesis, and a decrease in overall transcription, ribosome number, and chromosomal replication.

Research has historically focused on proteobacteria, with *E. coli* as the primary model organism. However, the enzymes that regulate the stringent response in Firmicutes differ significantly from those in *E. coli*, leaving a large gap in our understanding of a vital stress signalling pathway in important human pathogens.

We are studying the *C. difficile* stringent response using a range of chemical-biology and genetic techniques, to identify the environmental factors that lead to induction, and determine the cellular processes it controls. Our work suggests the stringent response plays an important role in *C. difficile* adaptation to antibiotic stress, as well as survival through nutrient limitation.

A162

Investigating the elongation and division machinery of the obligate intracellular bacterium *Orientia tsutsugamushi*

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Abstract

Orientia tsutsugamushi (Ot) is a cytoplasmic-dwelling, Gram-negative obligate intracellular bacterium which causes scrub typhus, a potentially fatal disease endemic in many parts of rural Asia. As an obligate intracellular bacterium it shows substantial gene loss compared to free living counterparts. One pathway that has been particularly affected is the peptidoglycan biosynthesis pathway which is minimal in Ot and produces a peptidoglycan-like sacculus that cannot confer stringent rod shape to the bacterial cell. It was unknown how the bacterial elongasome and divisome systems, which regulate insertion of nascent peptidoglycan in rod shaped bacteria, were affected in this bacterium and that was the focus of the current study.

Immunofluorescence microscopy was used to determine the subcellular localisation patterns of selected proteins involved in bacterial elongation or division (FtsZ, FtsI, FtsQ, MreB and PBP2). We identified changes in these patterns at different stages of intracellular growth and following drug treatment to disrupt MreB polymerisation or peptidoglycan biosynthesis. Some proteins displayed unexpected localisation patterns, which warrant further investigation.

This work begins to explore how division and elongation are achieved, and may be regulated, in a minimal bacterial system. It has wider implications for investigating the host-pathogen interactions of Ot and the evolution of bacteria from free-living organisms to endosymbionts with highly reduced genomes.

A163

Uncovering the pathways regulating RNase P function and stability under stress in *Escherichia coli*

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Abstract

The regulation of RNA processing and modification pathways is crucial for accurate gene expression and bacterial survival under environmental stress. A key component of such pathways is RNase P, an essential ribonucleoprotein complex that catalyses the maturation of precursor tRNAs required for protein synthesis. Despite its ancient and conserved role in translation, the mechanisms regulating RNase P expression, assembly, and stability remain poorly understood, even in the model organism *Escherichia coli*. Since its discovery in the 1970s, several conditionally lethal mutants have been generated in bacteria to study RNase P function *in vivo*. Among these, the temperature-sensitive *rnpA49* allele in *E. coli* has been particularly valuable for investigating RNase P activity. This mutation destabilises the RNase P holoenzyme by disrupting the interaction between its RNA and protein subunits, resulting in reduced enzyme activity at elevated temperatures. Using this strain, we recently identified multiple spontaneous suppressor classes that partially restore viability under non-permissive conditions through distinct molecular mechanisms, including copy number increases of RNase P subunit genes and loss-of-function mutations in Lon protease and RNase R. This work builds on those findings by serially passaging representative suppressor lineages under sublethal stress to examine how early genetic responses influence long-term physiological adaptation. We aim to uncover additional pathways involved in RNase P expression, regulation, stability, and turnover, which remain largely unexplored in *E. coli*. This work links RNase P regulation to broader questions in microbial physiology and evolution, elucidating how E. coli maintains important RNA processing and modification pathways under stress conditions.

A164

Identification and characterisation of surface features/molecules involved in *Pseudomonas aeruginosa* biofilm formation using a multi-disciplinary approach.

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Abstract

Pseudomonas aeruginosa is a clinically important opportunistic pathogen well known for its ability to form biofilms, complex multicellular communities that contribute to chronic infections and increased tolerance to antimicrobial treatments. Central to biofilm resilience is the extracellular polymeric substance (EPS) matrix, a heterogeneous network of polysaccharides, proteins, and extracellular DNA that provides structural support and protection. Despite its importance, the detailed composition and spatial organisation of the *P. aeruginosa* biofilm matrix remains incompletely understood.

This project investigates the structural and biochemical characteristics of *P. aeruginosa* biofilms and develops an optimised multimodal approach for their characterisation. Biofilms of the wild-type strain (PAO1), a polysaccharide-deficient mutant (*Δpsl Δpel*), and a mucoid overproducer of alginate (PDO300) were analysed using microscopy and biochemical assays. Quantitative crystal violet assays revealed pronounced variation in biofilm biomass, with PDO300 exhibiting up to sixfold greater accumulation compared with PAO1, while *Δpsl Δpel* produced negligible biofilm. Complementary microscopy (SEM, BacLight, and CLSM) confirmed and expanded upon these differences, showing denser surface coverage and increased matrix heterogeneity in PDO300 relative to the *Δpsl Δpel* strain.

These preliminary findings demonstrate that EPS composition has a strong influence on biofilm architecture and density, reinforcing the value of our comparative, multimodal analysis. Ongoing work aims to refine non-destructive imaging of hydrated biofilms to preserve native structure and enable cross-validation with biochemical data. Ultimately, this study seeks to improve experimental methods for biofilm characterisation and inform the development of strategies to disrupt biofilm formation and enhance antimicrobial efficacy.

A165

Examining the Role of Stress Responses in *Staphylococcus aureus* Cystic Fibrosis Infection

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Abstract

Staphylococcus aureus is one of the earliest pathogens to colonise and infect the cystic fibrosis (CF) lung. CF is a genetic disease affecting an estimated 105,000 people across 94 countries. Mutations in the host CFTR chloride channel lead to impaired mucus hydration and disrupted clearance. This altered lung environment blocks airways and promotes chronic respiratory disease. Stresses such as nutrient limitation, oxidative stress, antibiotic exposure, and acidic pH can activate a bacterial survival mechanism termed the stringent response, which promotes traits associated with chronic infection. Pathogens, including *S. aureus*, activate this pathway, which is mediated by the alarmone nucleotides guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp), to prioritise survival. The synthesis and hydrolysis of (p)ppGpp in *S. aureus* are carried out by the RelA/ SpoT Homologue (RSH) enzyme family, of which *S. aureus* has three. Upon production of (p)ppGpp, a global transcriptomic change is induced, leading to a slow–growth phenotype that promotes survival during stress. It is unknown about how external signals and host environments control stringent response activation in *S. aureus* during CF infection, however, we hypothesise that these signals will contribute to chronic infection and therapy resistance. Here, we aim to assess the requirement of (p)ppGpp and its three synthetase enzymes for survival of *S. aureus* in the CF lung. A greater understanding of this could aid in targeted treatment of recurrent CF infection.

A166

SpoIIIL is a forespore factor required for efficient cell-cell signalling during *Bacillus subtilis* sporulation.

Kaitlyn Coleman

ORCID iD[1], Danae Morales Angeles[1], Chimezie Progress Odika ORCID iD[2], Chris L. B. Graham

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Abstract

Bacterial endospores allow bacteria to survive stress, persist in the environment and seed new and recurring infections. During endospore formation, the mother cell and developing spore establish several essential signalling pathways that lead to activation of compartment-specific transcription and key steps in morphogenesis. In addition, communication between the mother cell and forespore involves a highly conserved membrane complex, called the A-Q complex, that, rather than directly regulating σ factor activity, plays a crucial role in sustaining forespore transcriptional potential. While SpoIIIL was previously identified as a putative A-Q complex component in *Bacillus subtilis*, its precise role remained unclear. Here, we demonstrate that SpoIIIL does not function in the A-Q complex but instead acts as a forespore-specific factor required for efficient cell-cell signalling, leading to late mother cell transcription.

A synthetic sporulation screen combined with cytological analysis indicated that *spoIIIL* has a genetic relationship with *murAB*, a paralog of *murAA*, required for efficient peptidoglycan precursor synthesis during sporulation; and a *spoIIIL murAB* double mutant is severely defective in spore cortex assembly. Subsequent investigations suggested SpoIIIL contributes to the activity of SpoIVB, a secreted forespore protease required to activate the signalling pathway for processing pro-σK to active σK in the mother cell. This, in turn, up-regulates the synthesis of peptidoglycan precursors required for cortex formation. Accordingly, the *spoIIIL* mutant exhibits delayed and reduced pro-σK processing and decreased accumulation of peptidoglycan precursors. Collectively, our findings redefine the role of SpoIIIL and provide new insight into the regulatory mechanisms controlling late mother-cell transcription.

A167

Comparative Lipidomic Profiling of Lesser-Known *Bacteroides* and *Parabacteroides*

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Abstract

Microbiome-derived bacterial lipids represent a pool of diverse, dynamic and often complex structures, a subset of which have emerged as key mediators in the microbe-host molecular dialogue. For instance, *Bacteroides thetaiotaomicron* (*B. theta*) produces bioactive *N*-acyl amines that can activate host pro-inflammatory cytokine production through TLR2 engagement. In addition, *B. theta*, among other Bacteroidetes, synthesise sphingolipids (SP), whose absence has been associated with intestinal inflammation in mice. However, the full diversity, functions and host interactions of gut bacterial lipids remain poorly defined.

Thus, the present study aims to comprehensively characterise the lipidome of several lesser known *Bacteroides* and *Parabacteroides* species, including *Bacteroides uniformis, B. salyersiae, Parabacteroides distasonis, P. johnsonii, P. goldsteinii* and *P. merdae*. Briefly, selected strains will be cultured in complex media (i.e. BHIS), harvested at mid-late log phase, and lipids will be extracted from bacterial pellets using isopropanol with internal standards representing major lipid classes. Extracts will then be analysed via LC-MS on an Agilent 6546 QTOF coupled to an Agilent Infinity II 1260 HPLC, with *B. theta* serving as a reference strain.

Preliminary analyses indicate the presence of a conserved 'core' lipidome, with notable variations in certain lipid sub-classes, particularly within the phospho- and sphingo-lipidomes.

By modulating environmental and nutrient conditions, we aim to expand this baseline lipidomic fingerprint. Observing changes in lipidomic signatures reflecting dynamic remodelling of the commensal lipidome – be it in composition or abundance – will grant insight into the how specific bacterial lipids contribute to maintaining intestinal homeostasis and mediate microbe–host communication.

A168

Silencing the Competition: Concentration-Dependent Bacteriostasis of Vibrionaceae by the Pseudomonas aeruginosa Quorum Sensing Signal HHQ

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Abstract

Microbial communities are shaped by interspecies chemical signalling and competitive interference through quorum-sensing (QS) molecules. Remarkably, although the concept of QS first originated from work done on the marine symbiont *Aliivibrio fischeri*, very little is known about cell-cell communication in the marine ecosystem. Marine sponges (Porifera) represent a rich chemical reservoir of natural bioactive compounds and understanding how QS operates in this niche would be an important ecological and translational advance. While more often viewed in the context of human disease and infection, *Pseudomonas aeruginosa* and *Vibrionaceae* species have been isolated from marine sponges sourced off the coast of Ireland. Here, we investigate the antibacterial effects of the *P. aeruginosa* QS signal 2-heptyl-4-quinolone (HHQ) against members of the *Vibrionaceae* family. Initial assays revealed that HHQ activity against *Vibrio* species is not binary but operates along a susceptibility gradient. While *Aliivibrio fischeri* was completely inhibited at low HHQ concentrations, species such as *V. alginolyticus* and *V. diabolicus* tolerated levels up to 100 µM. Comparative genomics revealed a second *cyd*AB operon in *V. parahaemolyticus*, also present in *P. aeruginosa*. However, deletion of the *cyd*AB genes did not sensitize the strain to 10 µM HHQ. Supernatant cross-feeding assays suggest an inducible, cell-associated mechanism of sequestration and/or detoxification, while prolonged exposure of *V. scophthalmi* to HHQ led to the emergence of spontaneous resistant mutants, indicating the potential for adaptive resistance under sustained selective pressure. Our findings reveal that HHQ functions as a species-specific antimicrobial in marine environments, with species-level tolerance evident in *Vibrionaceae*.

A169

Corticosteroids increase the expression of the Quorum Sensing genes in *Pseudomonas aeruginosa*

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Abstract

Background. *Pseudomonas aeruginosa* (PA) frequently colonises the lungs of individuals with chronic respiratory diseases such as Chronic Obstructive Pulmonary Disease (COPD), for which corticosteroids are the key treatment to reduce airway inflammation. Corticosteroids promote biofilm formation in PA (PMID40539031), contributing to the pathophysiology of COPD. Furthermore, corticosteroid treatment of Quorum Sensing (QS) mutants unable to synthesise QS Signalling Molecules (QSSMs) do not show an increase in biofilm biomass, leading to the hypothesis that QSSMs are implicated in mediating this phenotype. Our objective was to determine how corticosteroids impact QS pathways.

Methods. Luciferase-based reporter strains of the 3 QS systems were treated with corticosteroids fluticasone or budesonide and incubated for 20h at 37Cº in a TECAN plate reader, recording luminescence and OD every 30 minutes. Luminescence-based reporters of the QS mutants we also constructed to further study this interaction.

Results. Luciferase-based reporters of the QS systems showed a significant increase of the QS genes expression when treated with 1 µM fluticasone or budesonide. The knockout strains showed a dose-dependent response to the addition of exogenous QSSMs.

Conclusion. QS is involved in the modulation of biofilm formation of PA by corticosteroids. The knockout strains will be used to determine the concentration of the QSSM needed to restore the response to corticosteroids of the QS mutants. This could open the door to the utilization of QS inhibitors as adjuvants for the treatment of COPD, with the aim of reducing PA biofilm formation and the resistance it confers towards antimicrobials and the immune system.

A170

Investigation of GadR-Mediated Regulation of the GadT2D2 Glutamate Decarboxylase System in *Listeria monocytogenes* Using an EGFP-Based Transcriptional Reporter

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Abstract

Listeria monocytogenes (*L. monocytogenes*) is a major foodborne pathogen that remains a significant public health concern. A key factor contributing to its survival and infection within the host is its strong acid resistance. The GadT2D2 system plays a crucial role in acid resistance by converting glutamate to γ-aminobutyric acid, a reaction that consumes one proton and helps maintain intracellular pH. GadR, a newly identified transcriptional regulator, has been shown to be essential for activating the GadT2D2 system. In this study, we constructed an EGFP reporter by fusing the *gadT2D2* promoter region to the *egfp* gene, enabling real-time monitoring of GadR activity under different conditions. Our results showed that osmotic stress, in addition to acid stress, could activate GadR and induce fluorescence. The roles of the global regulators SigB and CodY in the activation of the GadT2D2 system were also investigated using this reporter. These findings will be presented together with a model accounting for the regulation of the GadT2D2 system by GadR.

A171

Investigating the Regulatory Mechanisms of Acid Resistance in the Food-borne Pathogen Listeria monocytogenes by Rewiring the Trehalose Operon Expression

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Abstract

Mga/RofA-like proteins are emerging class of transcription regulators that regulates virulence traits in pathogenic members of *Bacillota* (e.g. *Streptococcus*, *Enterococcus*, *Bacillus anthracis*). Their activities are thought to be modulated through phosphorylation, however the precise mechanisms are unknown. We have recently identified a Mga/RofA-like protein, GadR, in the important food-borne pathogen *Listeria monocytogenes*. GadR controls the expression of glutamate decarboxylase system (GadT2D2) and thereby regulates acid resistance, which determines gastric survival. Interestingly, GadR activates *gadT2D2* in rich media (brain heart infusion) but not in nutrient limited conditions such as minimum media (MM). Herein, we sought to understand how acid resistance is repressed in MM. For this, we engineered *L. monocytogenes* mutant stains by redirecting the sole trehalose metabolism operon *treBA* under the control of P*gadT2D2*, thus coupling trehalose assimilation with P*gadT2D2* activity. These mutant strains cannot grow in MM with trehalose supplied as a sole carbon source (MM-tre), confirming that P*gadT2D2* is inactive under nutrient limited conditions. Suppressors that restored growth in MM-tre acquired mutations in P*gadT2D2* or duplicated *treBA* operon. Interestingly, suppressor mutants are found from mutant strains already carrying *codY* mutations. On-going investigations are characterising how these mutations affect *gadT2D2* expression and acid resistance. These results will give insights on the regulatory mechanisms of virulence traits modulated by Mga/RofA-like proteins, inform food formulation to improve food safety, and potentially provide novel strategies for anti-virulence therapies development.

A172

Oh, Please! 5-Oxoproline (OP), a natural amino acid derivative, is consumed by Campylobacter jejuni via a series of DUF proteins.

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Abstract

5-Oxoproline (OP), a natural amino acid derivative, is generated from the spontaneous cyclisation of glutamate and glutamine *in vivo*. Historically, OP has been viewed as a waste product which is toxic to bacteria. However, since the discovery of a bacterial 5-Oxoprolinase, which converts OP to glutamate, its possible role as a nutrient source has emerged, yet the basis of its assimilation is unknown. Here, we characterise an operon comprising three proteins of unknown function that are putatively involved in the transport and utilisation of OP by *C. jejuni*. DUF969/979 appear to form an OP transporter which may represent a new sub-class of the divalent anion-sodium symporter (DASS) family. DUF2891 encodes a cytoplasmic protein with no clear function, yet our experiments demonstrate all three genes are essential for the utilisation of OP. Through phylogenetic analyses, we identified these DUF proteins to be largely absent in *Campylobacter* species other than *C. jejuni,* where it is conversely ubiquitous. We also scanned over 6000 reference genomes, representing the available breadth of all bacterial species, revealing homologues of DUF969/979 in ~700 species (>10% of all bacteria), indicating the ability to import exogenous OP is common. Our findings provide new insights into the nutrient acquisition strategies of *C. jejuni*, expanding current understanding of its metabolic plasticity, as well as presenting a widespread bacterial capacity for OP transport and utilisation.

A173

Carbon environment modulates metabolic phenotype and antibiotic susceptibility in mycobacteria

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Abstract

Non-tuberculous mycobacteria (NTM) are primarily environmental bacteria capable of causing significant disease in humans, particularly those with underlying immune or respiratory dysfunction. Incidence of NTM infection in the UK is increasing and is projected to increase further due to the increasing effects of climate change. Treatment of NTM infections is complicated by high resistance to antibiotics necessitating long term multidrug treatment for no guarantee of cure. The successful use of bedaquiline, developed for treatment of multidrug resistant *Mycobacterium tuberculosis*, for treatment of NTM infections has shown that metabolism and energetics is a promising target for antibiotic development. However, the effect of available carbon sources on metabolic fitness is not fully understood.

Here we show that simple modifications to the carbon environment of standard Middlebrook 7H9+OADC growth media produce distinct metabolic and physical phenotypes without impact on viable growth. We further show that these differences alter susceptibility to a range of antibiotics including bedaquiline. This work demonstrates that drug resistance is linked to the metabolic adaptability of NTM.

A174

Three's a crowd – Endosymbiosisin a Tripartite System

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Abstract

The living of one organism within another, endosymbiosis, has evolved independently multiple times across the tree of life and often includes interactions between vastly unrelated organisms. This phenomenon is central to our understanding of the evolution of emergent complexity in modern life, medicine, ecology and agriculture. In natural settings endosymbioses occur in the context of complex and diverse ecosystems. However, when studied it is often limited to overly simplistic models that revolve around one-on-one interactions between two organisms, obfuscating much of the complexity and diversity seen in natural settings. For our understanding of endosymbiosis to progress, more nuanced models need to be developed. *Paramecium bursaria* has emerged as a model for the study of primary and secondary endosymbiosis in eukaryotes. Its symbiosis consists of the ciliate host housing multiple species of algae and providing them with organic nitrogen (amino acids) in exchange for photosynthetic products (maltose). *P. bursaria* can hold multiple different species of symbiont simultaneously, the experimental opportunities this presents are yet to be explored. We propose expanding the *P. bursaria*-algae model to include the study of these naturally occurring tri-partite systems whereby *P. bursaria* plays host simultaneously to microalgae from either *Chlorella* or *Micractiunium* and the picoalga *Choricystis sp.* in what can be described as dual-algal systems. By increasing the number of selfish entities that simultaneously co-habit the host, we hope complexity seen in nature can be mirrored in a tractable system.

A175

Beyond reactive species: why does cold plasma kill bacteria?

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Abstract

INTRODUCTION: Cold plasma can eradicate bacteria in suspension and biofilms. Reactive oxygen species, like hydrogen peroxide (H_2O_2), are often cited as the main mediators of these effects. Thus, controlling H_2O_2 generation in cold plasma devices is desirable to optimise bactericidal efficacy. However, a systematic approach to optimisation must account for the multitude of factors that influence H_2O_2 generation.

METHODS: The Taguchi design of experiments (DoE) approach was used to investigate the effect and relative importance of four plasma jet operating parameters on the H_2O_2 concentration, [H_2O_2]: the treatment time, the treatment distance, the helium gas flow rate and the frequency of the power supply.

RESULTS: The DoE analysis identified the percentage contributions from each of the parameters above. The Taguchi model was able to predict the optimum combination of parameters to maximise and minimise the [H_2O_2] and this was validated by experiment with acceptable accuracy. The H_2O_2 maximised plasma (78.8 µM predicted) could inactivate Gram negative *E. coli* within 300 s but failed to eradicate Gram positive *S. aureus*. The H_2O_2 minimised plasma (62.8 µM predicted) killed *S. aureus* but lost its activity against *E. coli*.

IMPORTANCE: Cold plasma parameters could be optimised to control [H_2O_2] in the treated sample using DoE. Cold plasma bactericidal activity varies between species and broad-spectrum activity could not be predicted using [H_2O_2] alone, indicating that kill is only partially mediated by H_2O_2. This may highlight the effect of other cold plasma agents, e.g., short-lived species and electric fields, which necessitates further investigation.

A176

Comparative lipidome profiling of sulfonolipid-producing gut bacteria

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Abstract

Sulfonolipids are a unique class of bacterial membrane lipids characterised by a sulfonic acid headgroup and capnine backbone that are produced primarily by members of the phylum Bacteroidota including environmental *Cytophaga* species and gut associated *Alistipes* and *Odoribacter*. Sulfonolipids have recently emerged as bioactive compounds with a potential role in the microbiome-host molecular dialogue. For instance, certain sulfonolipids have been shown to interact with Toll-like receptor 4 (TLR4), dampening LPS-induced inflammation. Similarly, outer membrane vesicles (OMV) containing immunomodulatory sulfonolipids secreted from *Alistipes timonensis* and *Odoribacter splanchnicus* have been shown to suppress proinflammatory cytokines and enhance IL-10 production, suggesting a protective role for these lipids in the host. However, knowledge of sulfonolipid biosynthesis, diversity and function remains limited.

Thus, in the present study we aimed to comprehensively characterise the lipidome of known sulfonolipid-producers (*Alistipes* spp. and *Odoribacter* spp.) and suspected producers (*Butyricimonas virosa* and *Gabonibacter* chumensis). Bacteria were cultured in Brain-Heart infusion broth supplemented with L-cysteine and haemin (BHIS), lipids were extracted from bacterial pellets at mid-log phase using isopropanol that was spiked with representative internal standards. Extracts were then analysed via LC-MS on an Agilent 6546 QTOF coupled to an Agilent Infinity II 1260 HPLC. *Bacteroides thetaiotaomicron*, a non-sulfonolipid producing bacteria was included as a reference control.

Preliminary lipidomic analysis indicates that all strains share a ‘core’ lipidome, with subtle differences noted in sulfonolipid, phospholipid and *N*-acyl amine lipid profiles. Future work will examine how environmental and nutritional stressors influence lipid composition.

A177

AFM to probe structures involved in *Clostridium sporogenes* germination

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Abstract

Bacterial spores are the dormant and environmentally resilient forms of some infectious bacteria, including *Clostridium botulinum*, that allow them to persist through unfavourable conditions. Through the process of germination, which is triggered by environmental cues, bacterial spores release vegetative cells that can produce toxins. There is a complex interplay of proteins and structural changes that the spore undergoes as part of this process. *Clostridium sporogenes* is a non-pathogenic spore former with high degrees of similarity to other pathogenic clostridia, making it an ideal model organism for studies on germination. Atomic Force Microscopy (AFM) is a type of scanning probe microscopy that can recreate the detailed topography of biological samples and measure local changes in mechanical properties under a variety of experimental conditions. AFM has been used to resolve defined structures related to the key layers of the *C. sporogenes* spore, including the crystal lattice of isolated protein layers and large topographical features on the surface of whole intact spores. Currently, we are using fixed germination samples to investigate the topographical changes that occur at defined time points during germination in order to elucidate the roles of the individual proteins layers throughout this process and the appearance of the surface of the emerging cell.

A179

Multi omic's approach informs sustainable manufacture of Streptomyces-derived antibiotics

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Abstract

The United Kingdom has begun investing in sustainable manufacturing as part of comprehensive policy aimed at addressing climate change. Pharmaceutical production accounts for 5% of the UK's total carbon footprint; a significant portion of this is linked directly to feedstock used in bacterial fermentation biofactories. As natural producers of many antimicrobial compounds, *Streptomyces* bacteria have been optimised over the course of decades for drug manufacture via these large scale fermentations. Alternate feedstocks, like bread waste, have the potential to significantly reduce the environmental impact of antimicrobial production. However, current productions strains exhibit reduced antibiotic yield when using alternate carbon sources due to close regulation of carbon metabolism. To understand the mechanisms of this regulation, we use transcriptomic and metabolomic data to create detailed maps of several carbon metabolism and antibiotic pathways in *Streptomyces clavuligerus* production strains. The dataset allows us to visualise changes at each step in a pathway over time, and between carbon sources. This will inform the design of high titre production strains that can utilise environmentally conscious carbon sources, ultimately reducing the carbon footprint without sacrificing drug yield. This approach can also be adapted in other organisms to address sustainable drug manufacture across the industry.

Session : Mycobacteria: Challenges and progress

A180

Exploring a unique predator-prey relationship to uncover vulnerabilities in tuberculosis

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Abstract

The rise of drug-resistant tuberculosis calls for new methods for detecting and eradicating its causative agent, *Mycobacterium tuberculosis*. As a step towards improving detection, we sought to take inspiration from nature and determine how the only known predator of the Mycobacteriales, *Ca. Mycosynbacter amalyticus* binds its prey. As a first step to understanding this process, we have identified a new adhesin, ThioP, that mediates covalent attachment of *M. amalyticus* to its prey. We produced a recombinant form of ThioP fused to mCherry and, using fluorescent microscopy, showed that the adhesin forms localised foci on target bacteria, recognising sites of active cell wall biogenesis. We developed a series of *in vitro* adhesion assays and revealed that ThioP covalently binds the prey's peptidoglycan. This mechanism is supported by a 1.6 Å crystal structure we have solved of the adhesin domain. When considered in the context of our microscopy, this suggests for the first time that this material is solvent-exposed at discrete foci in mycobacteria. Conservation of this protein throughout the phylum suggests that forming a covalent bridge joining predator and prey peptidoglycan is a conserved predatory strategy, which we validated by replicating our findings using the distantly related *Nanosynbacter lyticus*. *In vivo* assays in this organism have also confirmed the essential role of ThioP in the predation process. This adhesin will be a useful tool for probing peptidoglycan structure and dynamics and exploring the predatory mechanisms of these understudied bacteria.

A181

The mycobacterial glycoside hydrolase LamH enables capsular arabinomannan release and stimulates growth

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Abstract

Mycobacterial glycolipids are important cell envelope structures that drive host-pathogen interactions. Arguably, the most important are lipoarabinomannan (LAM) and its precursor, lipomannan (LM), which are trafficked from the bacterium to the host via unknown mechanisms. Arabinomannan is thought to be a capsular derivative of these molecules, lacking a lipid anchor. However, the mechanism by which this material is generated has yet to be elucidated. Here, we describe the identification of a glycoside hydrolase family 76 enzyme that we term LamH (Rv0365c in *Mycobacterium tuberculosis*) which specifically cleaves α−1,6-mannoside linkages within LM and LAM, driving its export to the capsule releasing its phosphatidyl-*myo*-inositol mannoside lipid anchor. Unexpectedly, we found that the catalytic activity of this enzyme is important for efficient exit from stationary phase cultures, potentially implicating arabinomannan as a signal for growth phase transition.

A182

Biochemical characterisation of novel arabinan degrading and recycling enzymes in *Nocardia* species

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Abstract

The *Mycobacteriales Order* include several human pathogens such as M*ycobacterium tuberculosis* and *Nocardia brasiliensis*. Arabinogalactan (AG) is an important structural component of acid-fast mycobacterial cell walls. Previously, Jourani et al., (2022) identified two novel enzymes with α-D-arabinofuranosidase/α-D-arabinofuranase activity. Here, we have identified a third glycoside hydrolase which was biochemically characterised for novel β1,2-D-arabinofuranosidase activity. Furthermore, we show X-Ray crystallographic data of the 1st known enzyme/ligand complex using naturally occurring mycobacterial AG. We also provide new evidence of a recycling system in *Nocardia and Dysgonamonas gadeii for* D-arabinose into central carbon metabolism. Here, we show a potential enzymatic pathway involving an isomerase which converts D-arabinose into D-ribulose followed by a ribulokinase which then phosphorylates D-ribulose. We also briefly allude to an alternative pathway whereby D-arabinose is converted to arabinitol by a dehydrogenase which is then phosphorylated by a xylulokinase.

A183

Inhibitor Activity and Resistance Mechanism of Compound BTD7 against Mycobacterium tuberculosis

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Abstract

Background: Tuberculosis remains a global health, with the increasing prevalence of multidrug-resistant and extensively drug-resistant tuberculosis undermining the efficacy of current anti-tuberculosis drugs. In light of the stagnant progress in novel tuberculosis drug development over the past five decades, there is an urgent need for innovative therapeutic strategies.

Objective: This research aimed to characterize the efficacy of the novel compound BTD7 against *Mycobacteria and* to elucidate its mechanism of action.

Methods: The *M. tuberculosis* was subjected to the selection of spontaneous resistant mutants with different concentrations of BTD7. After three rounds of evolution, BTD7-resistant mutants were isolated and subjected to whole-genome sequencing (WGS). The results were confirmed through Sanger sequencing.

Results: The novel compound BTD7 demonstrated excellent *invitro* activity against *M. tuberculosis*H37Rv, with a minimum inhibitory concentration (MIC) of 0.0078125µg/mL. WGS of these mutants identified a common mutation in the essential gene *rv0902c*, which encodes the histidine kinase *PrrB,* Specifically, an A266C point mutation was identified, resulting in a non-synonymous amino acid change from Arginine to Leucine at position 266 (A266L) in the sensor histidine kinase PrrB.

Conclusion: BTD7 represents a promising new chemical entity targeting the sense of cell membrane of *M. tuberculosis*. Its potent activity, novel target, and effectiveness against drug-resistant strains warrant its further development as a potential component of new TB drug regimens.

A184

Spatial single-cell metabolic profiling reveals bystander cell adaptations in *M. tuberculosis* infected macrophage cultures.

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Abstract

Mycobacterium tuberculosis remains the leading cause of infectious death globally. Metabolic crosstalk between *M. tuberculosis* and its human host cell is a key component of its success, however, we still don't fully understand this mechanism. A prime example is the lack of understanding as to why some macrophages never become infected with *M. tuberculosis*. We hypothesise that signals from infected cells alter the metabolism of neighbouring bystander cells, creating conditions less favourable for infection. To test this hypothesis, we developed a highly sensitive mass spectrometry approach, enabling spatial single-cell metabolic profiling of the host macrophage with and without *M. tuberculosis* infection.

Single macrophages were capillary sampled using the Yokogawa SS2000, under confocal microscopy to preserve the spatial information. We successfully sampled single, live *Mycobacterium bovis* BCG (a containment level 2 surrogate for *M. tuberculosis*) infected macrophages, their uninfected bystanders and unexposed control cells. Applying an untargeted liquid chromatography-mass spectrometry (LC-MS) method, we successfully detected >10,000 metabolic features. In accordance with our hypothesis, bystander macrophages had a unique metabolic profile compared to the infected and control macrophages, suggesting cellular communication protected these macrophages from being infected. This pioneering study will help us to understand why some macrophages resist infection, and lead onto the development of host directed therapies as adjuvants to antibiotics.

A185

Unravelling Protein-Peptidoglycan Interactions in Mycobacteria: A Fluorescence-Based Pull-Down Assay

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Abstract

The peptidoglycan layer constitutes a vital component of the bacterial cell envelope, providing structural integrity and a protective barrier against environmental stresses. Peptidoglycan is a complex, insoluble polymer formed by a repeating β-1,4 linked disaccharide of *N*-acetylglucosamine and *N*-acetylmuramic acid, cross-linked by short peptides. Many proteins are known to interact with this structure, but measuring these interactions is technically challenging. Here, we modify a classic pull-down assay that harnesses peptidoglycan's inherent insolubility. Classical peptidoglycan pull-down assays are usually SDS-PAGE-based and thus limited in both throughput and sensitivity. To combat these limitations, we have developed a new assay amenable to a 96-well plate format using fluorescently labelled proteins. We have validated this assay using the lectin wheat germ agglutinin, which is known to bind peptidoglycan and bovine serum albumin as a negative control. We also show that other cell wall polysaccharides, such as arabinogalactan, do not interfere with the assay. Taken together, this assay provides a significant advantage over classical approaches. In the future, this assay will facilitate high-throughput screening of potential cell-wall binding proteins and inhibitors of these interactions, thereby enhancing the development of targeted interventions against bacterial infections.

A186

Novel Interferon-Gamma Release Assays Using Phase-Specific Peptide Pools and Label-Free Detection of IFN-γ for Tuberculosis Diagnosis

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Abstract

Background: Tuberculosis (TB) remains a major global health challenge. Current diagnostic tools are limited in distinguishing TB infection from active TB, hindering effective disease management. Developing accurate diagnostic tools that can stratify TB infection stages is crucial for improving prevention, control, and patient outcomes. This study aimed to identify novel *Mycobacterium tuberculosis* (Mtb) epitopes associated with specific TB stages and develop a sensitive, label-free IFN-γ electrochemical immunosensor platform for improved diagnostic precision.

Methods: Phase-specific Mtb antigens, including actively secreted proteins following bacterial replication and latency-associated proteins, were analyzed in silico for antigenicity (VaxiJen), MHC class I/II binding (IEDB), and cytokine induction potential (INFepitope, IL4pred). Selected epitopes were combined to design stage-specific IGRA assays. Separately, a novel electrochemical immunosensor was fabricated via one-step electropolymerization of polypyrrole (Ppy) with streptavidin, enabling dense and oriented immobilization of biotinylated anti–IFN-γ antibodies.

Results: High-potential actively secreted proteins eliciting both $CD8^+$ and $CD4^+$ T cell responses, along with latency-specific epitopes, were identified based on antigenicity and immunogenic potential. The selected epitopes will be experimentally validated. In parallel, a novel electrochemical immunosensor was fabricated via one-step electropolymerization with streptavidin, providing a controllable and highly reproducible method for optimizing biomolecule grafting by adjusting the potential and charge. Since streptavidin contains four binding sites for biotin, its random orientation on the electrode surface does not interfere with the proper orientation of biotinylated anti–IFN-γ antibodies, thereby maintaining efficient bio-recognition.

Conclusion: This study presents novel stage-specific IGRA and a versatile, label-free IFN-γ electrochemical immunosensor.

A187

The Buruli ulcer virulence factor mycolactone targets Vacuolar ATPase assembly and disrupts lysosomal function

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Abstract

Buruli ulcer is a neglected disease caused by *Mycobacterium ulcerans*. The sole virulence factor responsible for disease pathology is a cytotoxic polyketide lactone called mycolactone. We have shown that mycolactone inhibits Sec61-dependent co-translational translocation of secreted and membrane proteins into the ER. This causes proteostatic stress which is ameliorated by the autophagy pathway. The Vacuolar-ATPase is the protein complex responsible for acidification of endolysomal compartments and is essential for both canonical and non-canonical autophagy pathways. The core membrane proteins of the V-ATPase proton pump are resistant to mycolactone but the ATP6AP1 and ATP6AP2 needed for assembly of the complex are rapidly downregulated. Their loss leads to a gradual decline in lysosomal function and an inhibition of late stage autophagy. V-ATPase activity is not only required for lysosomal acidification but also plays multiple roles in control of intracellular infection, regulating phagocytosis, xenophagy and phagosome membrane repair. Targeting of the macrophage V-ATPase may represent a novel virulence mechanism that compensates for the loss of the ESX-1 secretion system in *M. ulcerans.*

A188

Material-dependent biofilm formation by Non-Tuberculous Mycobacteria: inhibitory effect of copper surfaces

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Abstract

Non-tuberculous mycobacteria (NTM) are natural inhabitants of water that can cause serious opportunistic infections acquired from the environment. Biofilm formation not only contributes to virulence but also plays a vital role in the persistence of NTM in the environment.

Biofilm formation by *M. avium*, *M. abscessus*, *M. chelonae* and *M. fortuitum* on materials commonly used in plumbing or medical devices (stainless steel 316L/304L, titanium, copper, silicone rubber, polyurethane rubber, PVC, CPVC, ABS, PET) were tested using disc coupons. New vs aged copper was compared for two strains. Biofilm cells were recovered from coupons by sonication and vortexing, then enumerated by cultivation on agar. Copper leaching into media surrounding coupons was measured and minimum inhibitory concentration (MIC) testing for copper performed.

Silicone rubber and polyurethane rubber yielded significantly more biofilm than almost all other materials for 4/8 and 5/8 strains respectively. 2/8 strains formed significantly more biofilm on plastics (except ABS) than metals. Copper yielded significantly less biofilm for all strains than all other materials. Aged copper was less inhibitory than new copper for one strain (but not the other), although still significantly reduced biofilm compared to other materials. Copper MICs ranged from 16mg/L to >512mg/L. In some cases, copper concentrations in media exceeded MICs yet biofilm persisted on coupons.

Copper surfaces significantly inhibited NTM biofilm compared to other materials, suggesting potential for controlling NTM colonisation in plumbing systems. However, this effect may diminish as copper ages. Nevertheless, these results have implications for selecting materials in plumbing and medical device design.

A189

Investigation of *Mb0222*, a gene essential for virulence in the causative agent of bovine tuberculosis, *Mycobacterium bovis*

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Abstract

Mycobacterium bovis is the causative agent of bovine tuberculosis (bTB). The *M. bovis* gene *Mb0222,* encoding a "double hotdog" hydratase, was identified as essential for virulence in a cattle transposon-sequencing screen. The orthologue in *Mycobacterium tuberculosis*, the cause of human TB, is Rv0216 and it has also been identified as essential for virulence in murine infection models. Furthermore, *Mb0222* was previously identified as being more highly expressed in *M. bovis* compared to *M. tuberculosis*. However, little is known about the precise function of *Mb0222*. In this study, we used the ORBIT recombineering method to successfully generate an *Mb0222* knockout in *M. bovis* AF2122/97. The knockout was confirmed by whole genome sequencing. To characterise the mutant, we performed a transcriptomics experiment under standard culture conditions (aerated, rolling culture) as well as under hypoxic stress conditions (standing culture). As compared to the wild type *M. bovis* AF2122/97, the *Mb0222* knockout displayed 106 differentially expressed genes (DEGs) under rolling conditions, and 62 DEGs under standing conditions. Amongst the DEGs were genes involved in lipid metabolism and carbon monoxide oxidation, systems that are important for persistence of the pathogen in vivo. Further characterisation of the mutant is ongoing, including in vivo virulence using a mouse model of infection.

A190

Killing Intracellular Pathogens with Antibiotic Nanocapsules

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Abstract

Antimicrobial resistance (AMR) is an increasing problem worldwide, with a predicted 10 million deaths a year from antibiotic resistant infections by 2050, and no new antibiotics have been approved for over 20 years. An alternative method for overcoming AMR is the repackaging of existing therapies to improve their efficacy and reduce toxicity. In particular, nanocapsules made of polymer (polymersomes) loaded with antibiotics can be used to effectively eliminate intracellular bacterial infection, without affecting uninfected cells. This project assesses whether anti-tuberculosis drugs can be encapsulated in polymersomes and whether encapsulated drugs can cause clearance of *Mycobacterium* species.

Polymersomes made of PEO-b-PCL loaded with doxycycline and rifampicin were made by nanoprecipitation, and characterised. Drug-loaded polymersomes were added to *Mycobacterium tuberculosis* cultures, both intracellular and extracellular, and bacterial growth and death measured. The effect of polymersomes on bacterial growth was compared to that of free antibiotic and polymer alone. The minimum inhibitory concentration of doxycycline and rifampicin against *M. smegmatis* were identified as 0.125 ug/ml and 16ug/ml respectively, and against *M. tuberculosis* were 4 ug/ml and <0.03125 ug/ml respectively. Polymersomes loaded with doxycycline and rifampicin exhibited inhibitory effect against intracellular and extracellular *M. tuberculosis.*

A 3D human-cell granuloma model was used to assess the uptake and effect of polymersomes in the granuloma environment. Again, polymersomes loaded with doxycycline and rifampicin showed inhibition of *M. tuberculosis* growth.

A191

Transcriptional Regulation of Mycolic Acid Desaturation in Pathogenic Mycobacteria

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Abstract

Tuberculosis is a prevalent infectious disease caused by *Mycobacterium tuberculosis (M. tb),* posing significant global health challenges. The unique long-chain fatty acids called mycolic acids of the cell envelope form a robust and impermeable barrier. Mycolic acids undergo key modifications such as desaturation and cyclopropanation, contributing to the cell wall structure, pathogenicity and drug permeability of *M. tb*. The desaturation step in the biosynthesis of mycolic acids is primarily carried out by the essential desaturases *desA1* and *desA2*. Double bonds introduced by these desaturases on the meromycolate chains are crucial for subsequent modifications like cyclopropanation, which fine-tunes the virulence. Recently, the transcription factor MadR (mycolic acid desaturase regulator) was identified as a repressor of *desA1* and *desA2*, modulating mycolic acid desaturation in response to cell envelope stress. MadR binds to acyl-CoAs, with only saturated species relieving its DNA-binding and repressive activity

This study investigates the impact of the loss of the *madR* gene in mycobacteria using *M. marinum* as the model organism. The *madR* mutant showed increased sensitivity to various stresses, altered cell surface hydrophobicity and significant impairment in biofilm formation, indicating weakened cell envelope integrity. Additionally, Transcriptional profiling of the *madR* mutant revealed upregulation of another desaturase, *desA1_1*, indicating an additional layer in the regulation of desaturation.

Together, these findings provide insights into how mycolic acid regulation influences mycobacterial cell envelope maintenance, survival and virulence, highlighting *madR* as a key regulator in lipid homeostasis.

A192

Investigating Non-Tuberculous Mycobacteria Serine/Threonine Kinases as Targets for Antibiotic Resistance Attenuation

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Abstract

Cases of pulmonary disease caused by non-tuberculous mycobacteria (NTM-PD), such as *Mycobacterium abscessus*, are increasingly prevalent globally. Poor host antibiotic tolerance and the pathogens' intrinsic resistance to most antibiotics limit the efficacy of the complex multidrug regimens currently used in the treatment of NTM-PD. It is therefore imperative that we investigate novel therapeutic strategies for treating NTM infections.

PknB is an essential serine/threonine kinase that is conserved across the *Mycobacterium* genus. This kinase has a regulatory role in cell wall synthesis and maintenance, including sensing and responding to antibiotic stress. Additionally, the cell wall of *M. abscessus* consists predominantly of non-canonical 3-3 peptidoglycan crosslinks formed by L,D-transpeptidases, rather than the "normal" 4-3 crosslinks catalysed by penicillin-binding proteins. This alternative form of peptidoglycan crosslinking contributes towards β-lactam resistance in this organism.

We are investigating the role of PknB and its influence on peptidoglycan cell wall synthesis, such as the balance between canonical and β-lactam resistant crosslinking patterns and how chemical inhibition of PknB may influence antibiotic sensitivity. If correct, repurposing existing kinase inhibitors and administering them in combination with β-lactams could present novel effective treatment regimens for *M. abscessus* and associated NTM infections.

This work presents a biochemical and biophysical characterisation of *M. abscessus* PknB and demonstrates synergistic effects of combining kinase inhibition with β-lactam treatment *in vitro*. Our findings provide an insight into PknB-mediated cell wall regulation and highlight kinase inhibitors as a novel strategy for tackling the growing challenge of NTM-PD by reversing β-lactam resistance.

A193

Investigating the Importance of *M. avium* and Alveolar Macrophage Iron Handling in the Context of Chronic Lung Disease

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Abstract

Nontuberculous mycobacteria (NTM) comprise a diverse group of environmental mycobacteria, distinct from Mycobacterium tuberculosis (*M. tuberculosis*) and *M. leprae*. They are opportunistic pathogens that cause chronic pulmonary infections, especially in individuals with underlying lung diseases, such as COPD, bronchiectasis, cystic fibrosis and asthma. A characteristic feature of these chronic lung conditions is iron dysregulation, with alveolar macrophages accumulating excess intracellular iron—creating a niche that may favour intracellular pathogens, like *M. avium*.

This study tests the hypothesis that *M. avium* exploits macrophage iron stores to enhance its intracellular replication. Using murine foetal liver-derived alveolar macrophages (FLAM) and primary human alveolar macrophages as in vitromodels for infection, it was shown that treatment with the iron chelator 2,2'-bipyridyl (BPY) significantly reduces *M. avium* intramacrophage replication, while iron supplementation promotes bacterial replication, all without compromising cell viability in culture.

Under defined iron-limited and iron-replete conditions, the iron-associated genes *bfr* and *bfd*, which are homologous to well-characterised iron storage and uptake genes in *M. tuberculosis*, exhibit temporally dynamic and iron-responsive regulation, indicating coordinated bacterial strategies to balance iron acquisition and storage, according to environmental availability. This makes them promising targets for gene-focused interventions (e.g., CRISPR-mediated deletions) to combat NTM burden in chronic lung disease.

These findings underscore the critical role of accessible intracellular iron pools in supporting *M. avium* persistence, and highlight iron chelation as a promising adjunctive therapy. This work advances our understanding of how altered iron metabolism contributes to NTM pathogenesis and opens avenues for host-directed therapies in chronic mycobacterial lung infections

A195

Geogenomic mapping of drug-resistant Mycobacterium tuberculosis from Ireland and overseas

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Abstract

This abstract is based on our recently published work titled *'Geogenomic mapping of drug-resistant Mycobacterium tuberculosis from Ireland and overseas'*, published in Genomics (Elsevier, 2025).

In this study, we performed an in-depth comparison of whole genome *M. tuberculosis* isolates collected in and overseas to explore the patterns of drug-resistant TB (DR-TB) in Ireland. Using hierarchical clustering, we assessed whether drug resistance in Ireland was acquired *de novo* or through the introduction of already-existing internationally circulating strains. The study focused on Lineages 2 and 4, the two dominant drug resistant lineages found in Ireland, which were found to belong to major international clusters differing in geographical distribution. SNP distance analysis revealed little evidence of widespread onwards transmission of DR-TB isolates nationally within Ireland, indicating that a key source of DR-TB in Ireland is translocation from countries where resistance has already been established. These findings highlight the need for ongoing genomic surveillance and heightened vigilance for cases linked to regions with high DR-TB burdens, given Ireland's extensive international travel connections.

Building on this work, we are currently investigating several international clades previously identified as being linked to Ireland and circulating in high-burden countries to elucidate their evolution and genomic characteristics.

A196

Elucidating the efficacy of gut microbiome-derived antimicrobial peptides as powerful therapeutic candidates against *Mycobacterium tuberculosis*

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Abstract

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb), is a worldwide health concern also known as the forgotten pandemic. It caused an estimated 8.2 million new cases and 1.25 million deaths in 2022 (WHO Global TB report 2024). Novel anti-TB regimens are of high priority to increase the efficiency of the current treatments, reduce the treatment duration, and combat antimicrobial resistance (AMR) in Mtb strains. Here we used a double auxotrophic Mtb strain, *Mycobacterium tuberculosis* H37Rv mc^{2}6206 to assess the activity of novel chicken gut-microbiome derived antimicrobial peptides (AMPs) - Cecacins (CHK peptides) 9, 55, 56, 77, 94 and 103 against Mtb. We investigated the activity of peptides alone and in combination with conventional antibiotics Bedaquiline (BDQ) and Isoniazid (INZ). We identified powerful anti-TB activity with minimum inhibitory concentrations of 1µg/mL for Cecacin-55, 2 µg/mL for Cecacin-77, 94, 103 and 4 µg/mL for Cecacin-9 and 56. All peptides exhibited low haemolytic activity against human erythrocytes and high therapeutic indices ≥30. They also showed low cytotoxicity against human cell lines in a resazurin-based cell toxicity assay conducted on human kidney (A498), lung (BEAS-2B), intestinal (Caco-2), liver (HepG2) and skin cells (HaCaT). Cecasin-77 was the most toxic against kidney (CC_{50} 175,7+/-5.1) and skin cells (CC_{50} 669,1+/-27.9) respectively. Additionally, synergistic activity was observed between BDQ and Cecacin9 (FICI=0.18), 56 (FICI=0.035), 55 (FICI=0.140) and 103 (FICI=0.254) whereas additive effects were recorded in combinations of BDQ/Cecacin-77 (FICI=0.51) and INZ/Cecacin-9.Overall, these findings underscore the great microbial specificity and therapeutic potential of AMPs in treating TB infection.

A197

Characterisation of cold shock proteins in *Mycobacteroides abscessus*

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Abstract

Cold shock proteins (csps) are ancient RNA-binding proteins that dynamically alter mRNA structure in response to cold stress, influencing transcription and translation at a global level. However, they are thought to play key roles in any response to stress that impacts coupled transcription-translation, and results in elevated levels of single stranded RNA, including antibiotic stress. This work aims to investigate the intrinsic mechanisms of antibiotic tolerance in the non-tuberculous mycobacterium *Mycobacteroides abscessus* with a focus on the role these csps may play.

M. abscessus has two characterised csps, CspA and CspB, which are well conserved across Mycobacterial species. We show that both *cspA* and *cspB* expression is significantly upregulated in response to translation-targeting antibiotics, including the clinically relevant drugs clarithromycin, azithromycin and amikacin. To investigate their possible importance in antibiotic resistance we are using a CRISPRi-based approach to deplete the proteins. We will discuss the effects of csp depletion on the overall growth kinetics, both in planktonic and a biofilm state, as well as the global transcriptional response to antibiotic translational stress.

A198

Exploring orphan CYPs in the human TB pathogen Mycobacterium tuberculosis

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Abstract

Tuberculosis (TB) is an infectious disease which kills over 2 million people in a year and has infected over 2 billion people worldwide. *Mycobacterium tuberculosis* (Mtb), the causative organism of TB has been found to have 20 cytochrome P450 genes which is a high number compared to its size. Microbial CYPs have been proposed to be used as in vitro models of drug metabolism in mammals as few have been described to mimic the CYP activation of drugs in humans. Only a handful of the 20 Mtb CYPs have been structurally and biochemically characterized. Further studies are needed to focus on the further biochemical characterization of orphan CYPs (which are the ones without a known function) as defining the contribution of individual genes can shed light on their involvement in resistance to antibiotics. TB treatments currently last for 6-9 months which can eventually lead to non-compliance and exacerbate drug resistant issues that is a major problem in TB control and anti-microbial resistance. This study explores the possibility of efficiently expressing and purifying an orphan P450 (Cyp138) which is suspected to play a significant role in the cellular portion of the infection process of tuberculosis. The development of a new drug is a multistep process that takes a long time and is exceptionally expensive to complete. The expression of protein and its purification play a key role in drug discovery as they can be used as drug targets to screen new drugs.

A199

Characterizing *Mycobacterium tuberculosis in vivo* phenotypes and the influence of metal ion abundance of local host environments during infection

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Abstract

Mycobacterium tuberculosis (*M. tb*) is the leading cause of death from a single infectious agent worldwide. Interactions with the host immune response play a critical role in shaping the phenotype(s) and survival of *M. tb* during infection. One mechanism used by the host is manipulation of the availability of metal ions - to sequester essential metal ions away from bacteria and also to increase the concentration of heavy metals, such as zinc, in the phagosome for antimicrobial effect. In response, *M. tb* has evolved various adaptative responses to withstand these stresses, including the differential regulation of metal efflux systems.

These interactions may contribute to the formation of phenotypically distinct subpopulations, including persister cells, antibiotic tolerant bacteria that are thought to play a role in long-term survival within the host during antimicrobial drug therapy. Understanding the micro-environmental pressures that lead to the emergence of these *M. tb* populations may be key to improving treatment outcomes.

This project explores how metal stress responses influence *M. tb* phenotypes represented in human sputa and from *in vivo* models of disease. The project focuses on zinc, using transcriptional profiling to define *M. tb* adaptations, and antimicrobial drug sensitivity assays to investigate the relationship between metal ion concentration and antimicrobial drug action for *M. tb*.

A200

Assessing the evolutionary landscape of MDR development in *Mycobacterium tuberculosis*

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Abstract

Introduction

Multidrug resistant TB is a major driver of AMR globally. To understand this urgent global health threat, we explored the evolutionary landscape of the lab strain H37Rv and the global dominant lineage of *M. tuberculosis* (L2), the Beijing strain.

Methodology

We used a stepwise *in vitro* evolution of resistance approach to investigate the determinants of resistance to rifampicin and isoniazid. Frequency of resistance to each drug (monoresistance) and in combination (MDR) were quantified. Evolved strains were phenotyped via MIC assays and MGIT growth curves, including time to positivity (TTP). Linking phenotype to genotype was undertaken using a bespoke DNA extraction method and Illumina sequencing.

Results

We found that the frequency of resistance to the secondary antibiotic is higher when arising on the background of pre-existing resistance to the first drug. The growth dynamics of resistant and drug susceptible ancestor strains were determined, with the TPP consistently higher in all resistant strains, marking differences in lag time that translates clinically to longer TB diagnostics time. Rifampicin resistant isolates displayed heterogeneity with clinically relevant mutations in *rpoB*, coupled with compensatory mutations across neighbouring genes. Deletions of *katG* are not seen clinically due to the high fitness costs, however amongst our isoniazid resistant isolates this cost was not seen in growth curve analysis.

Conclusion

This work highlights how *in vitro* evolutionary studies can predict evolutionary AMR and importantly, how associated fitness costs can lead to novel understanding and applications of diagnostic platforms for MDR TB.

A201

Divergent GH183 lineage in *Mycobacterium abscessus* uncovers toxin-like enzymes shaping mycobacterial cell-wall remodelling

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Abstract

Divergent GH183 lineage in *Mycobacterium abscessus* uncovers toxin-like enzymes shaping mycobacterial cell-wall remodelling

Eloise Nelson, Jean-Lou Reyre, Tracy Palmer, Patrick Moynihan, Elisabeth Lowe

Mycobacteria, including *Mycobacterium tuberculosis* and non-tuberculous species such as M. abscessus, remain major global health threats, causing over a million deaths annually[1]. A key determinant of their pathogenicity is their highly specialised cell wall, which contains complex D-arabinan-based polymers such as arabinogalactan and lipoarabinomannan that require dedicated enzymatic machinery for remodelling. The recently described GH183 family of glycoside hydrolases targets these D-arabinan polymers[2]. While two GH183 copies are conserved across mycobacteria, some species, including *M. abscessus*, encode multiple, highly divergent homologues. Several of these are predicted to be secreted toxins, as they are encoded adjacent to putative immunity proteins, whereas others display unusual C-terminal modular architectures whose functions remain unexplored. In this study, we heterologously expressed and characterised unique GH183 enzymes from M. abscessus that forms a phylogenetically remote cluster relative to all previously described GH183s. Our findings reveal unanticipated diversity within the GH183 family and suggest that these enzymes may have distinct roles ranging from potential virulence factors to housekeeping cell wall remodelling enzymes, providing new molecular insights into mycobacterial cell wall maintenance.

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A202

Impact of climate change of mycobacteria and host response to infection

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Abstract

Non-tuberculous mycobacteria (NTM) are environmental microorganisms increasingly linked to opportunistic infections, particularly in tropical and subtropical regions. As global temperatures continue to rise, changes in environmental conditions may alter bacterial growth, persistence, and virulence.

To investigate these effects, cultures were grown across a temperature range (15–43 °C) and under different metal supplementation conditions (Fe_2O_3 and $Al(OH)_3$). This was followed by growth quantification, colony morphology assessment, and whole-genome sequencing. Growth was strongly influenced by temperature, with strains from the Mycobacterium tuberculosis complex (MTBC) growing best between 20–35 °C, whilst NTM species (*M. kansasii*, *M. intracellulare*, *M. gordonae*, and *M. fortuitum*) maintained robust growth up to 43 °C. *M. abscessus* showed marked sensitivity to metal exposure; Fe_2O_3 enhanced growth, whereas $Al(OH)_3$ reduced it and induced a shift from rough to smooth colony morphology.

Genomic analyses revealed that BCG exhibited minimal genetic variation under these conditions. In contrast, *M. abscessus* showed more extensive changes, particularly in membrane-associated and metabolic genes: a short-chain dehydrogenase allele was absent at lower temperatures and under metal exposure, possibly linked to reduced virulence, whilst a CanB allele appeared only in metal-supplemented cultures, potentially supporting growth adaptation. Data will also be presented on virulence and development of antibiotic resistance in strains grown under different conditions.

These findings suggest that environmental factors - such as temperature and metal availability - can influence genotypic and phenotypic adaptation in NTM. Understanding these responses will be critical for predicting how environmental change may impact non-tuberculous mycobacterial ecology and pathogenicity.

A203

Investigation of β-Lactam Antibiotics as Treatments for Antibiotic-Resistant Mycobacterium tuberculosis

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Abstract

Each year, millions die from tuberculosis (TB), a chronic bacterial infection caused by *Mycobacterium tuberculosis* (Mtb). Worsening this epidemic, the growing prevalence of antibiotic-resistant Mtb strains, particularly those resistant to rifampicin, poses a critical threat to global TB control. Treatment success rates drop to approximately 50% following the emergence of rifampicin resistance, underscoring the urgent need for new therapeutic strategies. Recent evidence suggests that β-lactam antibiotics, traditionally considered ineffective against mycobacteria, may have unexploited potential in TB treatment. The clinical success of meropenem, a β-lactam antibiotic, has challenged long-standing assumptions and renewed interest in this drug class as an alternative therapeutic approach for treating antibiotic-resistant Mtb.

Therefore, we have evaluated the *in vitro* efficacy of a panel of β-lactam antibiotics against both drug-susceptible and rifampicin-resistant Mtb model strains. Comparative analyses were performed to assess antibiotic susceptibility, with the aim of identifying candidates that possess promising anti-mycobacterial activity and potential use in existing drug combinations. These findings contribute to the growing body of evidence examining the potential for β-lactam antibiotics to be used as viable options for the treatment of drug-resistant TB. Together, our findings highlight the possibility of repurposing existing antimicrobial agents to address this critical global health threat.

Session : Phage biology with a view to application

A205

(Ph)ight Against the Phage: A Challenge to Phage Therapy.

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Abstract

Pseudomonas aeruginosa is the leading cause of chronic lung infections in people with cystic fibrosis (CF). The rise of antimicrobial resistance has intensified the challenge of eliminating CF-associated chronic lung infections, prompting renewed interest in bacteriophage (phage) therapy as an alternative treatment to antibiotics. However, broader acceptance of phage therapy in CF healthcare requires a deeper understanding of phage-bacteria interactions within the complex CF-lung environment (CFLE). A key knowledge gap is how *P. aeruginosa* antiphage defence systems (DSs), systems that prevent phage infection in *P. aeruginosa*, operate within the CFLE.

This study investigated *P. aeruginosa* DS activity in response to the CFLE using both media designed to mimic the CFLE as a whole and individual components of this environment including mucin, polyamines, and branched-chain amino acids. Proteomic analyses showed that DS activity was influenced by the CFLE as a whole, as well as DS type. It was also observed that the Type II Restriction-Modification DS specifically conferred phage resistance under polyamine supplementation. AO Overall, this study provides an early investigation into how the CFLE influences *P. aeruginosa* DS activity, and highlights the complexity of DS activity, as well as the need for further investigation into DSs in the CFLE.

A207

From Structure to Therapy: Engineering Clostridioides difficile Phages to Overcome Barriers in Phage Therapy

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Abstract

Clostridiodes difficile is a major cause of hospital-associated infection and recurrent antibiotic-associated diarrhoea, yet phage-based strategies remain understudied. Therapeutic development has been slow due to a lack of naturally occurring strictly lytic phages, high strain-specificity and incomplete understanding of their infection mechanisms. This knowledge gap hinders our understanding of phage-host interactions, and while previous research has provided some insights into structure and function, further investigation is necessary.

To address these challenges, we established a versatile phage engineering platform that enables precise genetic modification of two C. difficile bacteriophages. Using this system, we introduced a protein tag to facilitate cargo delivery and are developing aptamer-based tools for live imaging of infection dynamics. We also engineered the phage tail needle to understand its role in host recognition and final infection, and created a plaque-independent method for quantifying phage titres. High-resolution cryo-EM reconstructions provided complementary structural insights into tail architecture and contraction, helping to link molecular structure with infection mechanism. Together, these complementary genetic, biochemical, and structural approaches offer new mechanistic insights into *C. difficile* phage biology and open the way for the rational design of optimised therapeutic phages.

A208

Synergistic Effect of Bacteriophage and Antibiotics Against Biofilm Forming *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa, a gram-negative bacterium, an opportunistic human pathogen is considered as a major cause of nosocomial infection and major causes of morbidity and mortality in immunocompromised patients. This bacterium has the capacity to create biofilms, which are extremely stable communities that helps to develop and maintain an infection. Biofilms are one of the reasons because of which treatment of pathogens is getting difficult. With lack of new antimicrobial agent use of bacteriophage for treating infection cab become a best alternative approach The primary focus of this study is to isolate lytic phages from sewage sample and examine the synergistic effect of phage and antibiotic over biofilm forming *Pseudomonas aeruginosa* isolates. Total of 1500 clinical specimens were processed for culture. Out of these, 288 specimens showed significant growth of *Pseudomonas aeruginosa*. In total 74.8%multi drug resistant and 31.94% biofilm producing isolates were identified. Out of total biofilm producer 78.26% were found to be multi drug resistant showing that formation of biofilm is one of the reasons for drug resistivity. Bacteriophages were isolated from sewage water sample and examined for its efficacy towards biofilm producing isolates. Out of 10, Pa_KB_19 showed effective result by eradicating biofilms also, when mixed with antibiotic, Ciprofloxacin (3µg), the phage enhanced the bactericidal activity of antibiotic as the combination was effective against the biofilm isolates which were initially resistant to Ciprofloxacin of 5µg concentrations. Thus, the combination of these bactericidal agents can be very promising for future use.

A209

Phage therapy to treat Group A Streptococcus in Necrotizing Soft Tissue Infections

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Abstract

Necrotizing Soft Tissue Infections (NSTIs), are rapidly progressing, life-threatening diseases where conventional antibiotic treatments often fail due to extensive tissue damage, microbial resistance and necrosis. *Streptococcus pyogenes* (Group A Streptococcus), commonly referred to as 'flesh-eating bacteria', are a leading cause of NSTIs. This project aims to develop targeted phage-based therapies against *S. pyogenes* addressing the critical need for alternative and more effective treatment options.

Given the scarcity of known phages infecting *S. pyogenes*, with only one confirmed lytic phage isolated to date, we have adopted a multi-faceted strategy, capitalising on recent advances in phage biology. Our strategy includes: isolating novel phages from diverse clinical and environmental sources; developing machine learning tools to identify novel phage satellites for potential use in targeted gene delivery; and mining a custom phage protein database to discover candidate endolysins for in vitro validation.

We have established a comprehensive biobank of clinically relevant *S. pyogenes* strains and built a database of prophages and phage satellites. In vitro validation of the lytic activity of candidate endolysins is underway against clinical strains. Notably, we have successfully isolated phages active against seven different *S. pyogenes* strains, including five different clinical isolates and are currently characterising their genomic diversity, host range and therapeutic potential.

Our findings advance our understanding of *S. pyogenes* phage-host interactions and contribute to the broader goal of designing the next-generation of safer, more effective phage-based treatments for NSTIs.

A210

Chimeric infective particles expand species boundaries in phage-inducible chromosomal island mobilization

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Abstract

Some mobile genetic elements spread among unrelated bacterial species through unknown mechanisms. Recently, we discovered that identical capsid-forming phage-inducible chromosomal islands (cf-PICIs), a new family of phage satellites, are present across multiple species and genera, raising questions about their widespread dissemination. Here, we have identified and characterized a new biological entity enabling this transfer. Unlike other satellites, cf-PICIs produce their own capsids and package their DNA, relying solely on phage tails for transfer. cf-PICIs release non-infective, tailless capsids containing their DNA into the environment. These subcellular entities then interact with phage tails from various species, forming chimeric particles that inject DNA into different bacterial species depending on the tail present. Our findings illuminate the mechanisms used by satellites to spread in nature, contributing to bacterial evolution and the emergence of new pathogens.

A211

Leveraging the *Leuconostoc* phage-host relationship towards optimal starter culture design

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Abstract

The importance of *Leuconostoc* strains is widely recognized within the dairy industry. In milk, they play a crucial role in the formation of aroma and texture of certain dairy products. Despite this, bacteriophages of *Leuconostoc* have received little attention compared to those of *Lactococcus* and *Streptococcus thermophilus*, the main lactic acid bacteria (LAB) used in the composition of dairy starters. This lack of interest may be due to the erroneous belief that *Leuconostoc* bacteriophages are rare in the dairy environment and have minimal impact on starter functionality. To address this knowledge gap, 172 phages were collected across 34 Leuconostoc host strains and investigated in terms of diversity, spectrum of sensitivity, and impact.

A212

Genomic determinants of phage compatibility and resistance in *Enterococcus faecium*

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Abstract

In recent decades, *Enterococcus faecium* has emerged as an important opportunistic bacterial pathogen, causing urinary tract, bloodstream and wound infections, many of which are recalcitrant to treatment with last-line antibiotics.

Bacteriophages (phages) are viruses that prey on bacteria, but not human or animal cells. Phages target and kill specific bacteria within narrow host ranges, including antibiotic-resistant strains, highlighting their potential for therapeutic use in treating resistant infections. While phages with strictly lytic lifecycles are the preferred choice for therapeutic use, relatively few lytic phages targeting *E. faecium* have been described in the literature, reflecting challenges in their isolation. By contrast, temperate phages, which can exist in either a lytic or lysogenic state, are widespread in *E. faecium.* This potential to enter the lysogenic state (a dormant state where the phage integrates into the bacterial genome as a prophage) currently excludes temperate phages from therapeutic application, despite their widespread prevalence in *E. faecium* suggesting a potential untapped resource for new phage-based treatments.

This work integrates host and phage genomic data to determine how repressor similarity, integration-site preference, and resident prophages influence temperate phage infection outcomes in *E. faecium.* This mechanistic understanding will inform more rational and predictive approaches to assessing the feasibility of including temperate phages in phage cocktails targeting this important opportunistic pathogen.

A213

Dissecting Cell-Surface Recognition Mechanisms of Novel Enterococcal Bacteriophages.

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Abstract

Enterococcus faecalis and *Enterococcus faecium* are commensal gut bacteria that can become opportunistic pathogens, causing infections such as bacteraemia, endocarditis, and meningitis. These bacteria play a major role in the antibiotic resistance crisis, leaving limited effective treatments. Bacteriophages (phages) therefore represent a promising therapeutic alternative.

We isolated and characterised 55 phages from diverse environmental sources targeting clinical *E. faecalis* and *E. faecium* strains. Phage genomes were sequenced using the Oxford Nanopore MinION platform and classified phylogenetically based on their whole-genome sequences, leading to the identification of a novel putative phage genus. Host ranging analysis revealed that several phages displayed broad-spectrum infectivity across multiple enterococcal hosts.

To investigate the determinants of phage binding, we constructed isogenic *E. faecalis* OG1RF mutants with defined modifications in the Enterococcal Polysaccharide Antigen (EPA) motif. Efficiency of plating (EOP) assays using 12 representative phages demonstrated distinct substrate-binding specificities, indicating that phages exploit diverse recognition strategies. EPA was identified as a critical determinant for infection by most phages, though some displayed EPA-independent binding mechanisms.

Together, these findings reveal the diversity of cell-surface recognition among enterococcal phages and establish EPA as a major, but not exclusive, receptor. This work establishes a framework for systematic characterisation of phage–host interactions and supports the rational design of phage cocktails as potential therapeutics against multidrug-resistant Enterococcus infections.

A214

Prophage-encoded anti-phage arsenal of Serratia historic isolates.

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Abstract

Bacteria and their viral pathogens, bacteriophages (phages), co-exist in every environment, engaging in continuous cycles of co-evolution. In response to this selective pressure, bacteria have evolved numerous strategies to prevent phage infection, collectively referred to as anti-phage systems. These systems employ a wide array of diverse mechanisms, including phage DNA modification/degradation, cellular NAD+ depletion, and inhibition of protein synthesis. Anti-phage systems often cluster together within chromosomal locations defined as 'defense islands' or on mobile genetic elements (MGEs) such as prophages. This tendency has been extensively leveraged, leading to the discovery of novel anti-phage systems.

Here, we analysed a collection of 215 historic isolates originally used to define the genus. Using custom bioinformatic pipelines developed in the Mariano lab, we mined their genomes to characterise the repertoire of anti-phage systems encoded within their prophages.

Using our approach, we identified and validated 22 novel candidate anti-phage systems located within *Serratia* P2-like prophages. These systems, expressed under a constitutive promoter, were validated by heterologous expression in *Escherichia coli* followed by challenge with a panel of 150 lytic coliphages. Notably, several of the novel candidates exhibit an architecture typical of toxin-antitoxin systems. Using fluorescence microscopy and biochemistry, we provide initial insights on their mechanism of action.

Together, these findings expand the known diversity of prophage-encoded defences and lay the groundwork for future applications in synthetic biology, phage therapy, and microbial engineering. Ongoing efforts in our lab aim to fully elucidate the mechanisms of these systems to unlock their functional and translational potential.

A215

Improved transfermal delivery of bacteriophages with choline based ionic liquid

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Abstract

As the efficacy of antibiotics is decreasing due to the increasing spread of antimicrobial resistance, phage therapy has received renewed attention as an alternative to antibiotics. Skin infections, especially acne, severely impact the quality of people's lives and impose a significant economic burden. *Cutibacterium acnes*, the bacterial species responsible for acne vulgaris, exists mostly colonising pilosebaceous glands. Due to the barrier of the skin, traditional topical delivery methods can be inefficient against *C. acnes*. In this project we explore the possibility of developing a formulation based on ionic liquids (IL) to enhance the intra- and transdermal delivery efficiency of phages for the treatment of *C. acnes* infections. Choline based IL, especially choline-geranic acid (CAGE), can increase the permeability of the skin. CAGE was synthesized and characterized using NMR, TGA, and DSC, and preliminary antibacterial and anti-biofilm tests with CAGE have been conducted. After isolating, purifying, and characterizing a new *C. acne* phage YC_XY, its stability in CAGE was assessed. Short-term incubation (24 h) in 5% CAGE did not significantly reduce phage activity, suggesting that phage could be co-administered with CAGE solutions. Multiphoton microscopy was used to observe the penetration of fluorescently labelled phage particles into porcine skin treated with CAGE, which provided the initial evidence of the ability of CAGE to facilitate intradermal delivery of phage particles. This has been further confirmed by using measuring the depth of penetration of fluoresencently labeled phage particles in skin tissue sections prepared using a cryostat microtome.

A216

Application of liposome-encapsulated bacteriophages for the control of intracellular *Listeria monocytogenes*

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Abstract

Due to its ability to multiply at low temperatures and strong invasive capacity, *Listeria monocytogenes* easily causes fatal infections in pregnant women, infants, and immunocompromised patients, resulting in the highest hospitalization rate and the second-highest mortality rate among all foodborne pathogens. In addition, *Listeria monocytogenes* can rapidly replicate within host cells, evade the immune system and possess natural resistance to many common antibiotics, presenting significant challenges for treatment. Bacteriophage therapy as one of the main alternatives to the use of antibiotics has been actively investigated and successfully applied in the treatment of various bacterial infections, but the use of phages against intracellular pathogens is more challenging. Liposomes are often used for the efficient delivery and release of drugs and biologicals into target cells, which can be applied to phage delivery. The aim of this project was to encapsulate *Listeria* phage P100 in liposomes to enhance its stability in human cells and improve its bactericidal efficiency against intracellular *Listeria*. *Listeria* phage P100 was found to have desirable physical and chemical properties, with no loss of activity within the pH range 4-6 and in certain organic solvents (25% ethanol and 0.002% Tween-20). In this study, we prepared ionizable liposomal formulations with particle sizes of approximately 150–200 nm using microfluidic mixing technology. These formulations enhanced the ability of phage P100 to enter human intestinal epithelial cells (TC7 cells) and improved its stability within the cells.

A217

Characterization of novel Vibrio phages as biocontrol agents against *Vibrio alginolyticus* and insights into its phage-resistant mutant

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Abstract

Pathogenic bacteria are an ongoing threat to intensive agriculture, including aquaculture. With emergence of antimicrobial resistance (AMR), novel non-traditional antimicrobials are urgently needed to minimize the dependence on antibiotics. Bacteriophage (phage) therapy, which uses naturally occurring viruses to kill specific bacterium, is gaining interest and offers huge potential for targeted pathogen control in aquaculture. However, many challenges regarding stability and emergence of phage-resistance must be overcome.

Here, we isolated and characterized eight Vibrio phages against an emerging aquaculture pathogen *Vibrio alginolyticus* and studied their bactericidal and antibiofilm potency. We then used next-generation sequencing to understand how the *Vibrio* species may gain phage-resistance.

Our results indicated that most of the isolated Vibrio phages (7 out of 8) shared <40% genomic similarity with phages isolated elsewhere, suggesting a new phage genus. The phages were stable in different temperatures (4-40°C), pHs (3-10) and salinities (0-50ppt) up to 6h without significant loss in viability. Although individual phages had variable bactericidal efficiency and bacteria rapidly developed phage-resistance, phage cocktail formulations were highly efficient and significantly suppressed bacterial growth up to 15h, inhibited biofilm formation ($p<0.05$) and eradicated established biofilms ($p<0.05$). Sequencing confirmed absence of lysogeny modules, known toxins and AMR genes in 7 phages and presence of tRNAs and a putative anti-CRISPR (Acr) proteins in 2 most efficient phages. Though bacteria rapidly developed phage-resistance, we observed increased antibiotic sensitivity as trade-off which possibly resulted from defective efflux pump. Our findings support potential applications of Vibrio phages in aquaculture systems for minimizing burden of Vibriosis.

A218

Bacteriophages vs *Escherichia coli*: A Targeted Approach to Decontaminate Packaged Meat

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Abstract

Antibiotic resistance in pathogenic *Escherichia coli* poses a significant challenge to animal health and food safety. Bacteriophages can specifically target and lyse bacteria, showing promise as an alternative to antibiotics for pathogen control. This study reports on the therapeutic potential of two novel bacteriophages (8A and 9A) that were isolated from chicken/pig faeces using a livestock enteric *E. coli* isolate as the target/propagation strain. The bacteriophages revealed no lytic activity against 40 avian and porcine *E. coli* isolates, indicating an extremely narrow host range which may be attributed to the customised strain-specific isolation technique. The bacteriophages have identical capsids (119-120nm) and contractile tails (137-140/20-25nm). Notably, bacteriophage 9A produced halo plaques, suggesting a potential depolymerase activity against capsular *E. coli*. Based on their distinct plaque properties, the bacteriophages were combined and therapeutically tested in chicken mince and mince broth over 24h. In both food matrices, a single application of the bacteriophage cocktail at a combined multiplicity of infection of 10 reduced bacterial growth to undetectable limits, 2h post-treatment at 37°C. Bacterial regrowth was observed afterwards signifying potential resistance effects. A significant (p<0.05) bacterial reduction (~4 $\log_{10}$ CFU/mL) was observed at 4-8°C, over the experimental time. This data show that the therapeutic efficacy of the bacteriophages is food matrix- and temperature-dependent, with superior performance in broth at 4-8°C compared to solid mince at 37°C. Genome sequencing, growth kinetics, stability, formulation and testing in various infection models will further reveal the therapeutic potentials of these bacteriophages in food packaging.

A219

Archaeal viruses to control and reduce enteric methane production in ruminants

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Abstract

Methane is a potent greenhouse gas, responsible for over 25% of the global warming we experience today. In 2020, ruminant enteric emissions (predominantly from cattle) accounted for approximately 27% of global anthropogenic methane emissions. Our project aims to deliver a first-of-its-kind solution that targets methane production in ruminants by using naturally occurring viruses to selectively remove methane-producing archaea, which are responsible for up to 90% of methane generated. By harnessing these viruses, we aim to modulate the rumen microbiome, eliminating methane-producing organisms without affecting cattle welfare or productivity.

The culture and isolation of methane-producing archaea present significant challenges. Four archaeal species commonly found in ruminants; *Methanobrevibacter smithii*, *M. ruminantium*, *M. gottschalkii*, and *M. bryantii,* were cultured on simplified media under a range of anaerobic conditions to improve growth rates and maximise the potential for virus isolation. These methanogens were then used to enrich for archaeal viruses. Our results show that simplified media can support robust methanogen growth, enabling consistent cultures for downstream applications. Preliminary studies indicate that this approach can reduce methane production by a dominant archaeal species by up to 76%.

This proposed solution has the potential to make a significant contribution to reducing methane emissions from ruminants while maintaining cattle health and welfare.

A220

DNA packaging of Gene Transfer Agents

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Abstract

Gene Transfer Agents (GTAs) are small phage-like particles produced by some bacteria and archaea. GTAs package random fragments of host DNA into virus-like capsids, with no preference for their own genes, and transfer the DNA to recipient bacteria. The lack of specificity in packaging is thought to be due to unique properties of the packaging motor complex or terminase. Phage terminases are composed of a large terminase subunit (TerL) that carries out enzymatic activities (ATPase and nuclease) and a small terminase subunit (TerS) that regulates TerL and recognizes the viral genome. GTA TerL contains conserved amino acids that are predicted to be responsible for GTA specific properties such as unusually low DNA packaging density. GTA TerS has been found to lack the DNA binding domain present in viral TerS and this is thought to lead to a lack of target specificity. Our results show that the start site of *Rhodobacter capsulatus terL* is located downstream of the computationally annotated start codon and indicate that TerS stimulates TerL nuclease activity and reduces ATPase activity. Cryo-EM structural data indicate that GTA TerL and TerS subunits appear to form a $(TerSL)_2$ tetramer complex in solution, in the absence of DNA, and we propose that this could represent the elusive packaging initiation complex. Our data provide new details about how GTA DNA packaging occurs and insights into the differences and similarities to their phage ancestors.

A221

Discovery of Zorya IV, a Novel Anti-Phage Defence System Subtype

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Abstract

Bacteriophages (phages) are viruses that prey on bacteria. Phages outnumber bacteria in every environment, driving the evolution of a vast arsenal of anti-phage defence systems. Since 2018, hundreds of novel systems have been discovered. One such mechanism is the Zorya defence system. To date, Zorya systems have been identified in three distinct subtypes, all sharing the conserved core components ZorA and ZorB. Structural analyses revealed that ZorA and ZorB assemble into a 5:2 rotary motor reminiscent of those that power flagellar rotation, type IX secretion, gliding motility, and Ton-dependent nutrient uptake. In Zorya types I and II, the effector proteins ZorD and ZorE, respectively, mediate the anti-phage response through nickase activity.

In this study, we report the discovery of a fourth subtype, Zorya IV, which retains the core ZorA–ZorB motor but encodes two additional components, ZorH and ZorI, that lack detectable nuclease domains. Native expression of Zorya IV confers resistance to diverse phages in Pseudomonas aeruginosa, and all four components are required for full activity. Ongoing work aims to dissect the contribution of each gene through single and combinatorial knockouts, to determine the molecular activity of ZorH and ZorI, and to establish whether the Zorya IV defence mechanism follows an abortive-infection strategy, as observed in other Zorya subtypes.

These efforts will help elucidate how distinct effector modules associate with the conserved ZorA–ZorB motor to diversify anti-phage mechanisms and broaden the protective spectrum of the Zorya family.

A222

Engineering Synthetic Lytic Phages to Combat *Clostridioides difficile* Infection

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Abstract

Despite significant national efforts to control Clostridioides difficile infection (CDI) leading to a fourfold decrease in cases between 2007/8 and 2013/14, the disease has continued to persist up until recently at these levels. A combination of recent data showing, for the first time in 15 years, an increase in CDI cases, alongside emerging resistance shrinking the already small pool of effective antimicrobials, demonstrates the need for alternative therapies to be explored and developed.

Discovered over 100 years ago, the untapped potential of bacteriophages (phages) for therapeutic use is only starting to be explored in the Western world. Due to temperate phages more commonly facilitating horizontal gene transfer in addition to the benefit of superinfection immunity conferred upon the bacterial host, lytic phages are seen as a more suitable candidate for therapeutic use. To date, only temperate phages active against C. difficile have been isolated, providing a significant barrier to future therapy against CDI.

As efforts to isolate naturally occurring lytic phages have so far proven unsuccessful, genetic engineering may offer a viable alternative. We aim to create synthetic lytic phage, overcoming this barrier and potentially paving the way for a future lytic phage cocktail product. The integrase and CI-repressor genes were determined to be the two genes essential for lysogeny, allowing integration and stability of the prophage within the lysogen. Knockout mutants were therefore created of the temperate phage, phiCD1801, to attempt to remove the ability to form lysogens. Characterisation of these mutants will be presented.

A223

'PanPhage': A Unified Model & Approach Integrating Phage and Bacterial Genomics for Predicting Host–Phage Interactions

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Abstract

Understanding bacteriophage–bacterium interactions is critical for developing effective phage therapies, particularly in veterinary and clinical contexts where host range prediction remains a major challenge. Previously, we developed predictive models for 31 bacteriophages and 301 E. coli isolates from canine urinary tract infection (UTI) samples. Each phage-specific model, built using random forest algorithm, utilized bacterial pangenome gene presence/absence as input features and predicted an interaction "Score", reflecting the difference in bacterial growth with and without phage exposure. Although these models achieved low root mean square error (RMSE) values, their reliability decreased for "biased" bacteriophages that infected only a limited subset of E. coli strains, due to imbalanced training data.

To address this limitation, we developed a unified 'PanPhage' model that integrates both bacterial and phage genomic features. Phage genomes were sequenced and encoded as gene presence/absence data, which were combined with the bacterial pangenome to form a joint feature matrix. Using gradient boosting, this single model predicts interaction scores across all phage–bacterium pairs simultaneously. Compared to updated individual phage models built with the same approach, the 'PanPhage' model demonstrated improved accuracy (lower RMSE) across both bacteriophages, with the greatest gains observed for biased bacteriophages. To evaluate generalizability, we further implemented Leave-Group-Out Cross-Validation (LGOCV) by excluding either an entire phage or bacterial sequence type (ST) during training.

This integrative approach highlights the value of combining phage and bacterial genomic contexts to enhance predictive modelling of host–phage dynamics, with direct implications for targeted phage therapy design.

A224

Phages and phage-derived proteins for *Proteus mirabilis* biocontrol

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Abstract

Proteus mirabilis is an opportunistic Gram-negative uropathogen responsible for a substantial number of catheter-associated urinary tract infections, especially in long-term catheterised patients. The globally increasing rates of antimicrobial resistance necessitate the search for new alternatives to antibiotics. Bacteriophages and phage-derived proteins, such as endolysins and depolymerases, have emerged as promising antimicrobial agents for the prevention, control, and treatment of bacterial infections. Several new *Proteus* bacteriophages were isolated, and it was demonstrated that phage particles can be immobilised on the surfaces of silicone urinary catheters treated with cold plasma, thereby creating an antimicrobial coating. It was further demonstrated that the anti-biofilm activity of a depolymerase-containing *Proteus* phage is enhanced by co-administration with cold-plasma-activated water, and that the synergistic effect of this combination is dependent on the order of treatment. Two *Proteus* endolysins and a depolymerase were identified and characterised, and the antibacterial properties of these enzymes were demonstrated. It was also shown that mortality in *Galleria mellonella* infected with *Proteus* could be reduced by the application of the purified depolymerase alone. Finally, a new machine-learning tool was developed to predict depolymerase genes in bacteriophage genomes.

A225

Analysis of detoxified E. coli strains for bacteriophage production

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Abstract

Characterised bacteriophage (phage) production strains are a vital, yet understudied, aspect in the pipeline of clinical phage therapeutics and their use in human clinical settings. In order to develop an appropriate production host for coliphage, a pair of clinical isolates of uropathogenic *E. coli* (UPEC) were chosen from a set of 314 canine and human urinary tract infections (UTIs). Selection of isolates were made according to criteria that would apply for production hosts across all bacterial species. These include absence of: antimicrobial resistance genes, predicted active prophage regions and, ideally, phage defence systems. For coliphage, lipopolysaccharide is a contaminant, as it is present in the host gram-negative cell wall, with the lipid A (endotoxin) component an inflammatory moiety, which triggers septic shock in humans. Two isolates, meeting the earlier criteria, were genetically modified to substantially reduce their endotoxin activity. This simplifies the often costly clean-up methods pre-administration. The two isolates selected were also chosen as they had a 'broad phage range', together able to produce 81/100 phage tested. Ongoing research is investigating how to broaden the phage range of these isolates further, such as receptor addition. Overall, the work supports the concept of a minimal strain set capable of producing safe phage preparations to treat the diversity of UPEC infections.

A226

Freeze-dried bacteriophage formulations against *Pseudomonas aeruginosa* infections.

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Abstract

Antimicrobials, formerly hailed as “miracle drugs”, are the cornerstone of modern medicine and indispensable in treating and preventing infections in humans, animals and plants. However, due to the rise of antimicrobial resistance (AMR), infections that were once easily treatable with first-line antimicrobials are becoming increasingly difficult to manage. This poses a major threat to global health with significant economic consequences. Most notable are the ESKAPE pathogens, a group of six highly virulent and antibiotic-resistant bacteria that are responsible for the majority of nosocomial infections. An ESKAPE pathogen of critical clinical importance is *Pseudomonas aeruginosa,* which is known for its intrinsic and acquired resistance to antimicrobials and ability to form a biofilm.

Bacteriophage therapy is one of the most promising alternative treatments for bacterial infections. With the resurgence in confidence in phage therapy, the formulation of bacteriophage products for therapeutic application is necessary for commercial circulation. However, bacteriophages often lose activity during formulation and storage, especially in suspension. This study investigates lyophilisation, or freeze-drying, as a method to preserve phage viability in a solid state for long-term storage at ambient temperatures.

A range of *P. aeruginosa* bacteriophages were chosen based on their lytic ability of PAO1 and retained their activity post-lyophilisation. Results showed that cryoprotectants were necessary to improve both phage activity and product stability during the freeze-drying process and subsequent storage. The freeze-dried product was subsequently incorporated into a hydrogel formulation as a potential treatment option for wound and burn infections caused by *P. aeruginosa.*

A227

Hi-C resolved rumen viromics improves host assignment and ecological insights

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Abstract

Bacteriophages, viruses that infect bacteria, shape microbial communities, including the rumen microbiome, yet assigning them to hosts in complex environments remains challenging.
We applied proximity-ligation (Hi-C) metagenomics to rumen contents from twelve dairy cows across four pasture-based diets to recover genome-resolved viral sequences alongside microbial metagenome-assembled genomes (MAGs) and plasmids. Because Hi-C captures physical interactions at the time of sampling, we contrasted these contact-based host links with in silico prediction (iPHoP), which leverages historical signals (e.g., CRISPR matches). We annotated genomes to catalogue auxiliary viral genes (AVGs) and to identify antimicrobial resistance (AMR) genes in their genomic context.
We resolved a large rumen phage catalogue and established thousands of virus–host links, including numerous prophages. Hi-C and iPHoP showed broad concordance on shared assignments, with Hi-C adding time-specific interaction evidence. Ecologically, viral productivity decreased as host abundance increased (consistent with kill-the-winner dynamic among rarer taxa), whereas dominant hosts exhibited higher lysogeny (aligning with piggyback-the-winner dynamics). AMR genes predominantly localised to bacterial chromosomes or plasmids, while carriage on viral genomes was rare, suggesting phage impacts on AMR act mainly via predation on ARG-carrying hosts rather than viral mobilisation of ARGs.
With Hi-C capturing phage–host interaction dynamics, its host assignments can inform putative hosts for closely related rumen phages in external datasets, including uncultivated viruses represented only in reference databases.
A Hi-C, host-resolved framework offers a practical route to characterise rumen phage–host networks and responsibly extend host annotations to related and uncultivated phages, supporting future ecological and applied studies.

Session : Vaccine platforms and gene therapy vectors

A228

Production and characterisation of antigenically stable enterovirus A71 genotype C4 VLPs in *Pichia pastoris*

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Abstract

Enterovirus A71 (EVA71) poses a serious threat of potentially lethal neurological disease, especially in infants and young children across Asia-Pacific region. During EVA71 replication, particles lacking viral genome termed empty capsids (ECs) are formed alongside infectious virions. ECs are assembled from 60 copies of three structural proteins, VP0, VP3 and VP1 arranged on an icosahedral lattice.
ECs produced in heterologous systems are termed virus-like particles (VLPs) and absence of genome makes VLPs non-infectious, safe and suitable as vaccine candidates. While initially antigenically indistinguishable from virions, ECs can rapidly expand at moderate temperatures. The conformational changes involved can result in loss of antigenic sites that may be required to elicit protective immune responses.

Previously, stabilising mutations were selected in EVA71 genotype B2, which increased the antigenic stability of the particles. Here, we introduced a single mutation (K1162M) into a recombinant expression system to produce stabilised C4 EVA71 VLPs in *Pichia pastoris*. Unlike wild-type VLPs, the recombinant stabilised C4 EVA71 (rsVLPs) maintain a native conformation (NAg) as indicated by reactivity with a selective antibody. The results showed that 90% of rsVLPs produced in *P. pastoris* are in NAg conformation and have a conversion temperature of approximately 42˚C. Negative stain EM demonstrated that rsVLPs retain icosahedral morphology, with particles ~32 nm in diameter.

The generation of these stabilised EVA71 VLPs in a yeast expression system demonstrates the potential for development of highly immunogenic, antigenically stable EVA71 VLPs, which could be used as a platform for vaccine manufacture in the future.

A229

Developing a Vaccine Against Staphylococcus Aureus Using a Reverse Vaccinology Approach

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Abstract

Staphylococcus aureus is an opportunistic pathogen renowned for its ability to circumvent the host immune response to ensure its survival. As a persistent coloniser of 30% of the population, it is a leading cause of both community- and hospital-acquired infections with its clinical manifestations ranging vastly from mild skin infections to detrimental, systemic infections such as meningitis and endocarditis. Effective treatment against *S. aureus* has become increasingly sparse following the emergence of multidrug resistant strains, most notably methicillin-resistant *S. aureus* (MRSA) and more recently, vancomycin-resistant *S. aureus* (VRSA). The robust resistance mechanisms presented by *S. aureus*, coupled with its complex pathogenesis and versatility, accentuates the demand for novel strategies to combat it. Despite decades of ongoing research, there is still no commercially available vaccine against *S. aureus*.

By utilising a novel priority antigen discovery pipeline, we aim to rectify the current lack of therapeutic strategies through the development of a safe and efficacious anti-*staphylococcal* vaccine. In silico analysis has allowed identification of a panel of 100 putative vaccine candidates, based on predicted antigenicity and global cross-protection. To verify these predictions, the corresponding gene sequences were then cloned and recombinantly expressed for in vitro screening. The screen revealed several proteins with high immunogenic potential, triggering elevated levels of the pro-inflammatory cytokines IL-17 and IFN-γ.

By combining computational antigen discovery with experimental validation this study will ultimately define protective antigen combinations essential for development of the first successful vaccine against *S. aureus* and, in turn, relieve the universal health threat it proposes.

Session : Virus Forum: Adaptive immunity

A230

In Vitro Maturation of Avian B cells and Susceptibility to Different IBDV Pathotypes

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Abstract

Infectious Bursal Disease Virus (IBDV) is a major global threat to poultry production due to its immunosuppressive effects on the bursa of Fabricius, a critical organ for B cell maturation in chickens. IBDV infection significantly depletes B cells, resulting in compromised humoral immunity, poor vaccine responses and increased susceptibility to other pathogens in both infected and recovered chickens. The extent of immunosuppression varies with viral pathogenicity, age of bird and B cell susceptibility, though understanding virus-host interaction remains a key challenge. Our ongoing research aims to investigate how different IBDV pathotypes interacts with B cells at various developmental stages to drive phenotypic maturation.

Currently, a key limitation in IBDV research is maintaining viability and mature B cells in culture. Our *in vitro* system uses different combinations of cytokines such as IL-4, IL-6, IL-21 and BAFF along with CD40L to drive maturation of naïve B cells isolated from the Bursa. Initial results demonstrated this approach promotes B cell viability while reducing immature marker Bu-1/chB6. While investigation of antibody production through immunoglobulin switching is ongoing, we aim to infect B cells at different maturation stages with various IBDV phenotypes to assess viral replication, cell viability and inflammatory response This study will comprise of IBDV strain: PBG98 (mild and doesn't infect B cells), D78 (a common vaccine strain that infects B cells in Bursa) and UK661 (very very virulent strain that induces systemic infection. This study will support ongoing projects and collaborative studies aimed at optimising vaccine development and understanding B cell-virus dynamics.

A231

Development of animal-free serology assays to assess measles immunity

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Abstract

Measles virus (MeV) is a highly contagious morbillivirus, which spreads through aerosols, initially infecting the respiratory tract before spreading throughout the body leading to systemic infection. Typically, symptoms include a high fever, cough, runny nose and a characteristic rash all over the body. MeV is also capable of causing severe disease and complications, such as pneumonia and encephalitis, and in some cases can even be fatal. Fortunately, the measles-mumps-rubella (MMR) vaccine is estimated to have prevented 17.1 million deaths worldwide between 2000 and 2014, with measles elimination achieved in several countries around the world. However, following a recent drop in vaccination rates, measles reemerged in the UK in 2018, with case numbers increasing year-on-year. In 2024, 2911 laboratory confirmed measles cases were observed in England. This resurgence of measles has been mirrored globally with an estimated 10.3 million MeV infections during in 2023.

Following the re-emergence of MeV in the UK, coupled with declining MMR vaccination rates, it has become increasingly important to understand population immunity to MeV to inform and direct public health interventions to the areas required. However, current assays can suffer from lab-to-lab variation, often impacted by small differences in the reagents used in different laboratories. Therefore, to address these variations, we have developed an animal-free ELISA and an animal-free VSV-based pseudovirus neutralisation assay to assess MeV immunity in a cohort of patient sera using chemically defined reagents. We believe that by adopting these chemically defined assays we will be able to improve inter-laboratory consistency.

Session : Virus Forum: Innate immunity

A232

Immunomodulation of innate immunity by vitamin D increases host resistance to mosquito-borne virus infection

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Abstract

With the growing challenges of climate change, mosquito-borne arboviruses are expanding their geographical range and public health impact. Understanding the factors that determine host susceptibility to infection remains critical, particularly in the absence of effective vaccines or antiviral treatments. Infection begins in the skin during mosquito feeding, where early host responses, such as inflammation, strongly influence disease outcome.

This study investigates whether vitamin D, a key immunomodulator, alters skin susceptibility to arboviral infection. We found that administering vitamin D at the inoculation site one week before infection markedly enhanced host resistance. This effect correlated with robust upregulation of type I interferon (IFN) stimulated antiviral genes in response to viral RNA. Importantly, vitamin D mediated protection was lost in IFN receptor deficient mice, demonstrating dependence on type I IFN signalling.

In vitro, vitamin D reduced viral load in both macrophages and fibroblasts. Notably, the antiviral effect in macrophages required type I interferon (IFN) signalling, whereas fibroblasts exhibited IFN-independent antiviral activity, indicating that vitamin D modulates distinct mechanisms across cell types

Together, these findings suggest that vitamin D modulates skin immunity and strengthens antiviral defences against mosquito-borne viruses

A233

Exploring the role of bovine IFIT1 and ZAP in the regulation of BPIV3 infection

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Abstract

Bovine respiratory infections often called Bovine Respiratory Disease Complex (BRDC) is a multifactorial disease and is one of the major reasons for loss in productivity and economic losses. One of the most important pathogens associated with BRDC is bovine parainfluenza virus 3 (BPIV3). Unbiased genome wide IFN screens exploring the antiviral response to human PIV3 (HPIV3) revealed the synergistic antiviral activity of human IFIT1 and human ZAP, two key antiviral restriction factors. Given the high degree of similarity between HPIV3 and BPIV3, we aimed to decipher the role of bovine IFIT 1(Interferon-induced protein with tetratricopeptide repeats 1**)** and ZAP (Zinc finger antiviral protein**),** both interferon stimulated genes (ISGs) known to have antiviral activities. Using CRISPR/Cas9 gene editing, we knocked out boIFIT1 and boZAP both independently and in combination in MDBK cells and infection is investigated using recombinant BPIV3-EGFP. The results obtained will give us the first glimpse into the role of bovine ISGs during infection with BPIV3 and can help in improving the greater understanding of role of bovine type I interferon system during infections.

A234

Inhibition of Zika virus by type I IFNα subtypes

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Abstract

Zika virus (ZIKV) is a re-emerging arbovirus, posing significant public health challenges due to its capacity to cause severe congenital abnormalities like microcephaly in neonates, and neurological disorders like Guillain-Barré syndrome in adults. At present, no specific antiviral therapies are available against ZIKV. The type I interferon (IFN) response is a critical component of the innate immune system, playing a pivotal role in controlling viral infections through the induction of interferon-stimulated genes (ISGs) with potent antiviral activities. Although IFNs are critical in controlling viral infections, the specific ISGs that effectively combat ZIKV remain unclear.

This project aims to evaluate the antiviral potential of the different human IFNα subtypes, with a particular focus on IFNα14 and its cancer-associated mutants (Q63H and F65L), which may exhibit altered antiviral activity. Recombinant IFNα subtypes were expressed in HEK293T cells and quantified using the HEK-Blue IFN-α/β reporter (Quanti-Blue) assay, while mutant IFNα14 proteins are being quantified using ELISA. Current experiments involve determining the 50% inhibitory concentration (IC50) of each IFNα subtype using plaque assays in A549 cells infected with the Asian lineage ZIKV strain PE243. Preliminary results confirm successful interferon production and quantification, and ongoing assays are assessing the differential antiviral efficacy of wild-type and mutant IFNα14 in comparison to other subtypes. This work will enhance the understanding of IFNα's role in modulating immune responses and could lead to new strategies for combating viral infections, tailored to individual genetic profiles.

A235

Influence of H5N1 2.3.4.4b Internal Gene Constellations in Modulation of Host Antiviral Defences in Bovine Cells

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Abstract

The recent spillover and spread of H5N1 clade 2.3.4.4b avian influenza viruses (AIVs) in dairy cattle is unprecedented and marks a concerning expansion of the virus's mammalian host range. AIVs typically replicate poorly in mammals, in part due to host antiviral defences such as type I and III interferon (IFN) responses and the induction of interferon-stimulated genes (ISGs). Understanding how H5N1 genotypes interact with bovine antiviral defences is important for assessing their replication potential in cattle and risk of onward transmission to other mammalian species. Using a panel of recombinant IAVs bearing internal gene constellations from diverse clade 2.3.4.4b genotypes, we assessed viral sensitivity to the bovine IFN-ISG response. We confirmed MX1 induction in bovine udder tissue following infection with recombinant A/Texas/37/2024 and demonstrated that all 2.3.4.4b reassortants were restricted by bovine MX1 and human MxA. Bovine-adapted viruses exhibited enhanced modulation of the IFN response, characterised by stronger host cell shutoff and reduced activation of IFN signalling compared to their avian ancestors. Segment swap experiments revealed that the PB2, NP and NS segments of bovine-derived B3.13 all contribute to this phenotype. Interestingly, genotype EA-2022-BB, which has infected fur farm animals, showed similar IFN modulation to the bovine-adapted viruses. Furthermore, most 2.3.4.4b viruses tested escaped human BTN3A3 restriction, one of the human genetic barriers to avian IAVs. Together these findings reveal that clade 2.3.4.4b viruses, including those that spilled into cattle, are evolving stronger suppression of host antiviral defences, increasing the pool of IAVs with zoonotic potential.

A237

Shared and Unique Features of Bat Interferon Lambda Proteins Underlying Antiviral Resistance

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Abstract

Zoonotic spillover events linked to bats like *Pteropus* species, such as the *Henipaviruses* Hendra and Nipah, highlights the continued need to study these hosts. It is thought that bats often remain asymptomatic during these infections that are often lethal for other hosts like humans and pigs. One popular hypothesis is that bats have unique immune adaptations to allow them survive such infections. While extensive research has been undertaken on characterizing bat antiviral immunity, little attention has been given to the type III interferon (IFN) lambda (λ) proteins, which are a specialized antiviral immune system functioning at barrier tissues like the airways. To address this gap, we use computational and *in vitro* approaches to better understand *P.alecto* IFNλ1 and λ3 in comparison to orthologues from other mammals like humans and pigs.

Although all IFNλs were produced and were functional, clear differences were observed in antiviral protection against a panel of model viruses in human and non-human cells within and between species. Bat IFNλ1 having the lowest activity across all cell types. Kinetic and thermostability assays showed similar activity profiles of all IFNλs tested, independent of protein glycosylation patterns as assessed by immunoblot. Sequence and structural analysis unveiled extensive diversity in bat IFNλs with clear evidence for IFN-receptor co-evolution. However, in attempts to validate our results in *P.alecto* PaKi cells, which were permissive to viral infection, they did not show any measurable response to IFNλ treatment. Altogether, our work suggests key shared and unique features of bat IFNλ proteins for further study.

Session : Virus Forum: Molecular & Cellular Biology

A239

The role of African Swine Fever core shell proteins in the nucleus during viral replication.

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Abstract

African swine fever (ASF) is a lethal haemorrhagic disease of pigs and wild boar causing mortality rates up to 100%. There is a lack of commercially licenced vaccines or treatments and therefore outbreaks of disease results in high economic loss for the pork industry.

ASF is caused by a large dsDNA virus (ASFV) that has a genome of 170 to 190 kbp, encoding more than 150 genes. ASFV primarily replicates in cytoplasmic viral factories, however previous studies have reported components of the virion core, and viral DNA within the host nucleus during infection. Despite this, the role of the nucleus in virus replication is controversial and the function of ASFV core proteins remains unclear.

A comparative analysis of the localisation of viral core proteins in infected cells and when transiently overexpressed was undertaken. A104R and K78R, two DNA-binding proteins both showed a strong localisation to the nucleus when individually overexpressed and during late infection. To assess their function, pull-down assays and mass-spec experiments were used to assess protein-protein interactions within cells overexpressing individual proteins.

During replication, multiple changes to the host nucleus were observed and therefore the localisation of nuclear bodies, nuclear speckles and other nuclear markers was analysed. During late infection, the number of nuclear speckles decreased whilst increasing in size, implying that speckles were coalescing. The relationship between nuclear core proteins and alterations to the nuclear architecture is now being explored to shed light on the role of African Swine Fever core shell proteins during viral replication.

A240

Characterising differential antiviral responses in cell lines permissive to foot-and-mouth disease virus infection.

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Abstract

A fundamental gap in knowledge for high consequence viruses such as foot-and-mouth disease virus (FMDV) is understanding the molecular basis of species-specific outcomes of infection. During infection, detection of viral double-stranded RNA and proteins stimulates the integrated stress response (ISR), leading to the formation of stress granules (SGs). However, this is counteracted by FMDV proteases cleaving key SG components including G3BP1. Concurrently, RIG-I like receptors (RLR)-mediated innate immune response (IIR) is activated, inducing interferon production and the transcription of antiviral genes. Both pathways are critical for controlling viral replication and likely influence disease outcomes. A key question is whether differential antiviral responses observed among cell lines permissive to FMDV correlate with the distinct clinical outcomes of infection in different mammalian species.

The study aims to establish the roles of ISR and IIR pathways in restricting FMDV replication in permissive cell lines derived from species either susceptible or resistant to disease: porcine (susceptible with clinical symptoms), African buffalo (susceptible without clinical symptoms), and human (non-susceptible). Experimental framework includes transfection with FMDV proteases and infection with FMDV, followed by molecular and cellular virology approaches, such as immunoblotting, confocal microscopy, and virus titration. Using these, we are uncovering the underlying mechanisms linking virus-mediated G3BP1 cleavage to IIR activation, and how it impacts the species-specific outcomes of FMDV infection.

Overall, this will generate fundamental knowledge of virus-host interactions and antiviral responses, besides providing a significant resource to the FMDV and livestock virology community, highlighting how intrinsic cellular properties influence viral sensing and replication.

A241

From Zoonosis to Pandemic: Evolutionary Refinement of Influenza A PA-X Host Shutoff Through Amino Acid Substitution.

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Abstract

The influenza A accessory protein PA-X mediates host shutoff, suppressing host gene expression to facilitate immune evasion and viral replication. While broadly conserved, PA-X exhibits sequence divergence across strains, reflecting adaptive evolution to distinct host environments. From the 1918 Brevig Mission strain with its hyperactive shut off efficiency to the more recent swine flu Pandemic strain (Cal09) with its comparatively moderate host shut off efficiency, understanding how these variations alter host interactions will provide mechanistic insight into viral evolution and potential indicators of pandemic emergence.

Initial comparisons examined PA-X from A/WSN/33 (H1N1), A/Brevig Mission/1/1918 (H1N1), a bovine H5N1 isolate (Tex24), and A/California/04/2009 (H1N1pdm09). HEK293T cells were co-transfected with a luciferase reporter plasmid and PA-X expression constructs to assess translational shutoff, and host mRNA levels were quantified by qPCR across a panel of transcripts. The Historic Brevig Mission and newer Tex24 PA-X variants exhibited comparable hyperactive shutoff efficiency, with WSN being less efficient and Cal09 being the least efficient but still significantly depleting transcript and reporter levels compared to the control.

Ongoing work focuses on Cal09 and Tex24 PA-X representing, respectively, a fully human-adapted pandemic strain and a lineage only beginning to cross the species barrier. Sequence–function analyses aim to link X-ORF amino acid substitutions with altered engagement of host transcripts. These findings highlight how incremental changes in PA-X recalibrate the host interactome and suggest evolutionary trends that could inform surveillance strategies and the prioritisation of host–virus interactions as potential targets for intervention, thereby contributing to pandemic preparedness.

A242

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

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Abstract

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

A243

Visualising faulty influenza virus gene products *in situ*

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Abstract

Defective interfering viral genomes (DVGs) are aberrant influenza virus replication products characterised by large internal deletions. During infection *in vitro,* high DVG abundance restricts virus replication and enhances the innate immune response, which correlates with less severe infection outcomes observed *in vivo*. Here, we present the development of fluorescent *in situ* hybridisation (FISH) methods designed to study how DVGs influence influenza virus spread within the host.

Specifically, we are developing single molecule FISH (smFISH) and Hybridisation Chain Reaction (HCR) methods as they allow for single cell, single molecule and multicellular analyses, respectively. These approaches, combined with commonplace plaque assays, offer a system to analyse DVGs across space and time. Since DVGs contain large internal deletions, smFISH and HCR probes sets were designed to bind either the central or the distal regions of the PB2 gene segment. When used simultaneously, these probes allow us to differentiate internally deleted DVGs from full length genes.

Preliminary tests show that the probe sets detect influenza virus RNA in infected cells and that individual DVG-producing cells can be identified. We are continuing to refine the probe sets to detect both individual DVGs and DVG-positive cells and optimise *in vitro* infection systems for our experimental work. This will allow us to study DVGs within spreading influenza virus infections and investigate their influence on viral population dynamics.

Overall, FISH offers promising methods to study the role of DVGs at the site of infection across different scales.

Session : Virus Forum: Vaccines & Antivirals

A245

Screening of orthologous Mx1 antiviral activity reveals host- and viral-dependent IAV restriction

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Abstract

Highly pathogenic avian influenza (HPAI) viruses, of H5Nx clade 2.3.4.4b, have caused a global panzootic since 2018. Outbreaks in farmed fur animals in Europe, marine mammals in South America, and dairy cattle in the US in recent years highlights the potential for these avian viruses to cross the species barrier and potentially adapt to a new mammalian host. Mx1 is an important restriction factor of avian-origin IAVs, targeting the NP in both the early and late stages of the viral replication cycle. In this study, we focused on understanding the antiviral activity of 18 diverse mammalian Mx1s on a range of avian-origin IAVs, some of which have successfully spilled over into mammals and some which have not been detected in mammals to date. The overall aims were to i) understand which host species express an antiviral form of Mx1 which may be applying a selection pressure to the virus and therefore potentially driving escape mutations to human MxA and ii) ask which avian IAVs are Mx1-resistant, and which NP residues define this, to help inform outbreak preparedness efforts for circulating IAVs. The panel of avian-origin zoonotic viruses [with glycoproteins from laboratory-adapted PR8 (A/Puerto Rico/8/1934)], were restricted by only certain Mx1 orthologues from the diverse panel overexpressed in MDCK cells. Interestingly, we observed strain-specific sensitivities for certain orthologs of Mx1, suggesting certain avian IAVs are better placed to escape this important antiviral defence and allows further studies to identify important viral determinants to Mx1 sensitivity/resistance.

A246

Targeting SARS-CoV-2 with Adaptable Miniproteins: A Proof of Concept for Pandemic Preparedness

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Abstract

The COVID-19 pandemic, caused by SARS-CoV-2, underscored the urgent need for preparedness against viral pandemics. This challenge demands a multidisciplinary approach that integrates fundamental science, computational modelling, and experimental validation to accelerate antiviral discovery.

We have developed an innovative platform that combines artificial intelligence and physics-based computational protein design, as well as experimental virology to enable the discovery, development, and validation of evolvable and rapidly adaptable antivirals. This platform can be readily applied to a wide range of viruses, including newly emerging pathogens and variants.

As a proof of concept, we focused on SARS-CoV-2. Using computational protein design, over 150 helical miniproteins targeting SARS-CoV-2 spike receptor-binding domain were initially generated. Of these, 51 were successfully expressed, and 24 demonstrated binding to the viral target - four with high affinity - all showing high thermal stability. To evaluate neutralisation capacity, we established *in vitro* neutralisation assays using lentiviral particles pseudotyped with the spike protein. Three miniproteins displayed strong neutralising activity (low IC_{50} values), and one exhibited exceptional potency.

Given the high mutation rates of RNA viruses, the emergence of resistant variants remains a major challenge in antiviral therapy. In ongoing work, we are assessing the impact of SARS-CoV-2 variants on miniprotein activity and exploring how these molecules can be rapidly re-engineered to maintain efficacy against evolving viral strains.

A247

Vaitera macrocarpa lectin: a lactose-binding protein that inhibits alphavirus infection *in vitro*

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Abstract

Introduction: Alphaviruses, such as chikungunya virus (CHIKV) and Mayaro virus (MAYV), have significant medical importance based on their consequences to patients, including persistent arthralgia. Nonetheless, there are no specific antivirals against CHIKV and MAYV. In this scenario, VML lectin, a protein isolated from the seeds of *Vaitera macrocarpa,* a native plant from the Brazilian Cerrado and the Amazon region, represents a compound to be investigated against alphaviruses, based on its reported biological activities, such as antibactericidal and pro-inflammatory. Here we evaluated the antiviral activity of VML against CHIKV and MAYV *in vitro*. **Materials and Methods:** Cell viability and antiviral activity were evaluated using concentrations of VML ranging from 200 to 1.56 µg/mL in Vero E6 cells. The viability was measured by MTT assay, and the viral replication rates were evaluated employing CHIKV-*nanoluciferase* and MAYV-*nanoluciferase*, two infectious clones containing a *nanoluciferase* reporter gene. Also, a time-of-drug addition assay was employed to assess the mode of action of the compound. **Results:** Data demonstrated that VML inhibited both CHIKV and MAYV, with a selective index of 12.4 and 6.3, respectively. Also, VML was active during both pre-treatment and post-entry stages of the viral life cycle, suggesting that it acted at multiple stages of virus infection. **Conclusions:** Our data show that lectins possess antiviral activity against CHIKV and MAYV, representing candidates for the development of future antiviral drugs against alphaviruses. Besides that, more analyses are needed to better understand its mechanism of action.

A248

Production of enterovirus VLPs in a *Pichia pastoris* derived cell-free system for point-of-care vaccine deployment

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Abstract

Over the past decade viral outbreaks, such as the Ebola epidemic and COVID-19 pandemic, have demonstrated the need for faster delivery of effective vaccines. Funded through the 100 Days Mission at CEPI (the Coalition for Epidemic Preparedness Innovations), our consortium is working to create virus-like particle (VLP) vaccine candidates in a yeast-based cell-free system that can be adapted to the self-contained MANGO (MANufacturing on the GO) device. The MANGO device aims to automate the production and purification of VLPs, is easily transportable, and can therefore operate at the epicentre for viral infection providing a route to faster outbreak control.

VLPs offer a safer vaccine alternative to traditional vaccines that require the production of live virus. As VLPs can be produced by expression of capsid subunits in heterologous expression systems there is no risk of viral genome packaging and therefore, no risk of viral infection. VLP exemplars for the project include poliovirus serotype 1 (PV1) the causative agent of poliomyelitis, and enterovirus A-71 (EVA-71) the agent for hand, foot and mouth disease (HFMD). We have previously produced both PV1 and EVA-71 VLPs intracellularly in the yeast *Pichia pastoris* (*Komagataella phaffii*). These are antigenically identical to virus and are therefore potential next-generation vaccine candidates. However, purification involves multiple steps including high pressure cell lysis, which is unsuitable for point-of-care production.

Here, we compare VLPs produced in both systems and assess key attributes such as antigenicity, morphology and yield.

A249

Comparison of immunogenicity and protective efficacy of aerosol and intramuscular immunisation with viral vectored vaccines encoding M1, NA2 and M1-NA2 in pigs

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Abstract

There is an urgent need for broadly protective influenza vaccines with the potential to reduce annual immunisation requirements, decrease seasonal infections, and lower mortality from pandemic strains. Pigs are a highly relevant large animal model for understanding influenza immunity as they are natural hosts for similar subtypes of influenza strains and share many physiological, anatomical, genetic and immunological similarities to humans.

We previously demonstrated that prime-boost aerosol immunisation of pH1N1 pre-exposed pigs with viral-vectored vaccines (ChAdOx2 and MVA) expressing the conserved internal matrix (M1) protein protected against lung pathology following H3N2 challenge.

While NA sequences evolve over time, they do so more slowly than hemagglutinin, making them a promising target for broader immunity. Here, we investigated whether adding neuraminidase (NA) to M1 (NA+M1) enhances protection compared to M1 alone or NA alone following either aerosol or intramuscular vaccine delivery in a prime-boost regime of ChAdOx2-MVA or ChAdOx2-ChAdOx2.

Protective efficacy was assessed by measuring lung pathology and viral load in nasal swabs and lungs. Antibody and T cell responses were analysed in blood and the respiratory tract. Our data indicate that viral-vectored vaccines expressing both M1 and NA provide superior protection compared to M1 or NA alone. These results offer valuable insights into the development of effective broadly protective influenza vaccines in a highly relevant natural host, the pig.

Keywords: influenza, vaccine, ChAdOx2, neuraminidase, aerosol, intramuscular

A250

Porcine influenza H3, H5 and H7 specific monoclonal antibodies

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Abstract

Pigs are natural hosts for influenza A viruses, with H1N1 and H3N2 subtypes co-circulating in both pigs and humans. Frequent bidirectional transmission between both species contributes to viral evolution and emergence of novel pandemic strains. Pigs are an excellent model for studying influenza immunity due to being immunologically, physiologically and anatomically similar to humans.

We previously demonstrated that pigs infected with the H1N1pdm09 virus generated monoclonal antibodies (mAbs) that recognised the same antigenic sites in hemagglutinin (HA) as human mAbs. Importantly, one of these epitopes was not detected by ferret antisera, which are routinely used in global influenza surveillance and vaccine strain selection.

Here, we generated mAbs against seasonal H3N2 virus or following sequential immunisation with the human quadrivalent inactivated vaccine, followed by single-cycle replication influenza vaccine candidates H5- and H7-S-FLUs.

Seven H3-specific, twenty H5-specific, and twelve H7-specific mAbs were generated and these were characterised for binding to recombinant HA proteins or to HA-expressing MDCK cells. We assessed neutralising activity and cross-neutralisation against different H3, H5, and H7 strains. Hemagglutination inhibition assays identified mAbs targeting the receptor-binding domain, while competition of binding of human anti-stem mAb MEDI8852 helped identify mAbs directed against the HA stem.

We showed that porcine H3 mAbs recognised key egg-adaptive H3N2 mutation similar to human antibodies, and that some of the H7 mAbs were directed against the HA stem. These porcine mAbs represent useful research tools for studying influenza virus evolution, exploring therapeutic antibody applications, and evaluating pharmacokinetics in a model that reflects human biology.

A251

PLATINUM(II)–PHOSPHINE COMPLEXES AS ANTIVIRAL CANDIDATES AGAINST ALPHAVIRUSES

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Abstract

Introduction: Chikungunya virus (CHIKV) and Mayaro virus (MAYV) are the etiologic agents of chikungunya and Mayaro fevers, respectively, diseases that cause hyperthermia, muscle pain, and skin rashes. There are no licenced antiviral drugs against CHIKV and MAYV infection. In this regard, metallic complexes with platinum(II) [Pt(II)] have previously demonstrated antiviral activity against several virus. **Aim:**Here we assessed the potential of new compounds in which phenylpyridine C^N cyclometallated platinum(II) was coordinated to different phosphines and chloride (Pt(II)(phpy)Cl(L), where L is PPh_3 (Pt(II)-1), PPh_2(Ph-COOH) (Pt(II)-2) or DMSO (Pt(II)-3), against CHIKV and MAYV infection. **Methods**: Dose-response assays were performed with Pt(II)-1, Pt(II)-2 and Pt(II)-3 in presence or absence of CHIKV or MAYV expressing the *nanoluciferase* gene for 24h. Additionally, time-of-addition assays were performed to investigate the effect of compounds on different stages of virus replicative cycle. Renilla-*luciferase* assay was performed to measure viral replication levels 24h post-infection and MTT assay for cytotoxicity. **Results:**Pt(II) compounds showed selective indexes (SI) for CHIKV and MAYV, respectively: Pt(II)-1=12.2 and 11.8; Pt(II)-2 >11.8 and 20; Pt(II)-3=14.7 and 20. Strong antiviral activities were observed on entry steps, all compounds inhibited > 60% of CHIKV infection, and > 50% for MAYV. **Conclusion:** Pt(II) coordinated compounds demonstrated an effective anti-CHIKV and MAYV activity *in vitro*, presenting potential as source of molecules for the development of novel antivirals against alphavirus.

A252

Unravelling Antigenic Diversity and Broadly Cross-Reactive Epitopes in H9N2 Avian Influenza Viruses.

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Abstract

Avian influenza H9N2 viruses are endemic across Asia, the Middle East, and parts of Africa, posing persistent threats to poultry health and of zoonotic transmission. They exhibit extensive antigenic diversity which is largely attributed to mutations within the haemagglutinin (HA) glycoprotein that alter antibody recognition. Antigenic drift among the circulating lineages, G and B, results in vaccine effectiveness being compromised. It is critical that cross-reactive epitopes, conserved region of HA that is recognised by antibodies across divergent strains, are identified to increase vaccine breadth and pandemic preparedness.

In this study, HA genes from representative G and B lineage H9N2 viruses were subjected to site-directed mutagenesis and rescued via reverse genetics to identify lineage-specific epitopes. Twenty recombinant viruses, each carrying a single amino acid substitution within the HA receptor-binding site corresponding to differences between the two lineages, were analysed. Four mutants exhibited a loss of haemagglutination activity with chicken red blood cells. Protein modelling indicated that these charge-altering mutations disrupt receptor-binding avidity, which may also influence antigenicity. Further evaluation of these residues through virus neutralisation assays and biolayer interferometry will help define their functional role in mediating cross-lineage immune recognition. These findings provide a basis for identifying broadly cross-reactive epitopes shared between the G and B H9N2 lineages, supporting the rational design of next-generation antigens for cross-protective H9N2 vaccines to safeguard global poultry populations.

A253

Efficacy of a novel antigen-decorated adenoviral vaccine platform against porcine respiratory coronavirus infection in a large natural host

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Abstract

Respiratory coronaviruses (CoVs) pose a significant threat to global health, where zoonotic spillover has resulted in three major human epidemics within recent decades. Many emerging CoVs can use livestock species such as pigs as reservoirs of infection, which can lead to severe economic losses. This highlights the urgent need for more broadly protective vaccines against porcine CoVs.

Porcine respiratory coronavirus (PRCV) infection typically causes mild disease but serves as a powerful model for acute respiratory coronavirus disease: interstitial pneumonia and lung pathology in infected pigs resembles that of human COVID-19 patients. Likewise, pigs are an ideal large animal model for vaccine development due to their physiological and immunological similarities to humans.

Here, we evaluated a novel adenovirus (Ad)-based vaccine platform using the DogTag/DogCatcher superglue system to generate several Ad-vector vaccine candidates. Ad-vectors encoding the PRCV135 Spike (S) and Nucleocapsid (N) proteins with surface decoration of PRCV135 Spike receptor-binding domain (RBD) conferred non-sterilising protection against PRCV135 challenge. Intramuscular immunisation with Ad-(S-N) RBD induced the greatest antibody responses and reduction in lung pathology and viral load compared to the other groups. This response was also cross-protective against other strains of PRCV and Transmissible Gastroenteritis Virus.

Based on this candidate, we also developed a multivalent cocktail of RBD-decorated Ad vectors targeting PRCV, porcine hemagglutinating encephalomyelitis virus (PHEV), and porcine deltacoronavirus (PDCoV) that generated an equally potent antibody response against all three pathogens.

This work demonstrates the potential of antigen-decorated Ad vectors as a platform for next-generation CoV vaccines.

A254

Harnessing PROTAC-Mediated Kinase Degradation for Next-Generation Antiviral Strategies

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Abstract

Antiviral drugs that directly target viral proteins are often virus-specific and prone to resistance, as shown by mutations emerging against the SARS-CoV-2 protease inhibitor Paxlovid, underscoring the need for broad-spectrum, durable strategies. Host-targeted approaches such as PROTACs, bifunctional molecules that harness the cell's ubiquitin–proteasome system to selectively degrade host proteins essential for viral replication, offer a potential solution.

Using a seasonal coronavirus model system, a panel of PROTAC compounds targeting various kinases, co-opted by viruses for their own replication, were assessed for antiviral activity. The PROTACs demonstrated potent antiviral activity while remaining non-toxic across multiple cell lines, and were effective at low micromolar concentrations, significantly reducing infectivity and viral RNA levels following treatment. Target kinase degradation was confirmed by Western blot analysis, and the effect was blocked in the presence of the proteasome inhibitor MG132, indicating proteasome-dependent activity.

Kinetic studies revealed distinct degradation profiles across the panel, suggesting different degradation efficacies or target mechanisms. While some PROTACs resulted in protein level decline within 4 hours and had sustained suppression with continued treatment, others showed more rapid but transient degradation, with partial recovery despite ongoing. Nevertheless, antiviral efficacy was maintained. The PROTACs outperformed conventional small-molecule kinase inhibitors targeting the same kinases, achieving greater viral suppression achieving greater antiviral efficacies.

Future studies will focus on mapping downstream phosphorylation and signalling changes and validating antiviral efficacy in kinase knockout systems. These findings support the development of kinase-targeting PROTACs as broad, host-directed antivirals with superior potency and durability compared to classical inhibition strategies.

A255

Inhibitors of the Wnt pathway reduce respiratory syncytial virus (RSV) growth kinetics.

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Abstract

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infection in infants and older adults. Host-directed therapies that target cellular signalling pathways such as Wnt/β-catenin could offer a complementary approach to direct antivirals by disrupting host processes required for viral replication and spread.

RSV infection upregulated Wnt pathway genes *CTNNB1* (1.5-fold) and *AXIN2* (5.5-fold), consistent with pathway activation. We evaluated two small-molecule Wnt inhibitors: iCRT14 (a β-catenin/TCF transcriptional antagonist) and ICG-001 (a CBP/β-catenin interaction inhibitor) at low and high concentrations. At low concentration only ICG-001 reduced RSV titres). At high concentration both iCRT14 and ICG-001 significantly attenuated viral growth kinetics at 48 hours post-infection (hpi) but showed no effect at 24 hpi. Concordant with lower viral titres at 48 hpi, expression of *IFNL* was reduced. Interestingly, treatment did not reduce measured inflammatory cytokines nor *ISG15* expression, indicating that Wnt inhibition lowered viral load without broadly suppressing these inflammatory or interferon-stimulated responses.

Pharmacological inhibition of Wnt/β-catenin signalling impairs RSV replication at later stages of infection in vitro, supporting a pro-viral role for Wnt activation. The differential effects of the two inhibitors and the concentration dependence point to target-specific mechanisms within the pathway. Targeting Wnt signalling warrants further exploration as a host-directed antiviral strategy against RSV.

A256

Predicting antigenic evolution of priority viruses from a single sequence

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Abstract

Understanding antigenic evolution in viruses is crucial for designing effective vaccines and drugs that can work against future variants or novel viruses. Traditional methods for determining antigenic evolution of virus proteins require a high volume of sequence data, together with relevant experimental results. The pandemic preparedness strategy against infectious viral diseases needs a redefined in-depth overview of virus antigenicity and site variation considering there would not be much related sequence data for the pathogen 'X' or novel pandemic-causing virus. We endeavor to predict antigenic evolution of an emergent virus by applying a pre-trained protein language model (PLM), ESM-2, on a single glycoprotein sequence, before comparing these predictive metrics with curated data from the protein sequence alignments and deep mutational scanning (DMS) experiments for the viruses that have validated experimental evidence such as SARS-CoV-2, HIV, and Influenza. The predictive metric, 'epistatic antigenicity', ranks higher for the antibody escape mutations, as evident in literature and curated database, compared to single amino acid effect on antigenicity, EVEscape (an approach that considers related viruses and protein structure), and DMS scores. For a novel virus, i.e., Crimean-Congo hemorrhagic fever virus, Bornavirus-1, and Marburg virus, this predicted antigenicity scores of the glycoprotein sequence determine sites and amino acid changes with high variation that may help escape antibody and low variation that may be targeted by therapeutic approach. The results demonstrate that PLM-probability and embeddings from a single sequence capture amino acid change probability and antigenicity at each residue—the key features to predict virus antigenic evolution.

BLOCK B

Session : AI-enabled Microbiology

B001

Using machine learning to investigate the molecular determinants of vector-borne transmission in arboviruses

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Abstract

Arthropod vectors enable virus host switching and emergence, with many viral families independently evolving vector-borne transmission. However, molecular adaptations underlying this dual-host lifestyle remain poorly understood. While virus-host interactions are mediated by proteins, extensive sequence divergence obscures evolutionary relationships in sequence-based analyses. Structural comparisons provide superior sensitivity for detecting homologous viral proteins since protein structure is more evolutionarily conserved than sequence. Recent advances in computational structure prediction enable analysis of millions of uncharacterized proteins, but leading platforms have not been validated specifically for viral proteins. Therefore, it is pertinent to benchmark the state-of-the-art protein structure prediction platforms to find the most suitable option for these pairwise structural comparison searches.

The four major platforms, ColabFold (AlphaFold2), AlphaFold3, Chai-1, and Boltz-1 were benchmarked using the nonstructural proteins NS1 and NS4A from Dengue and Powassan viruses. Platform performance was evaluated through similarity, consistency, and accuracy metrics using local distance difference test (LDDT) scores and template modelling scores (TM-scores). Predictions were then validated against experimentally determined cryo-EM structures where available.

ColabFold and AlphaFold3 significantly outperformed Chai-1 and Boltz-1 under the analysis conditions used here. All platforms showed reduced accuracy for the transmembrane protein NS4A compared to the globular protein NS1, reflecting inherent challenges in modeling disordered regions. Despite ColabFold's advantages for specific viral proteins, AlphaFold3 emerged as optimal for comprehensive downstream applications due to its superior consistency, accuracy, and capability for modeling diverse biomolecular complexes. These data will underpin the accuracy of structure-based phylogenetic studies used for understanding vector-borne virus evolution.

Session : Engineering (Micro)biology: Reprogramming microbes towards global solutions

B002

Modular Reconfiguration of Bacterial Signalling for Cancer Diagnostics

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Abstract

Bacterial two-component systems (TCSs) are powerful tools for synthetic biology, offering modularity and adaptability for diverse sensing applications. This study presents a novel diagnostic strategy by reconfiguring the *E. coli* EnvZ/OmpR TCS to detect cancer-associated metabolites. EnvZ, a membrane-bound histidine kinase (HK), and OmpR, a transcriptional response regulator, normally regulate porin expression in response to fluctuating extracellular osmolarity. By replacing the sensory domain of EnvZ with those from metabolite-sensing HKs, we have engineered chimeric receptors capable of responding to non-native signals.

These constructs will be expressed in *E. coli* EPB30, a mutant containing transcriptional fusions of *yfp* and *cfp* with *ompF* and *ompC*, respectively, to track the TCS output. Additionally, the *envZ* gene in EPB30 has been replaced with a kanamycin resistance cassette to prevent OmpR phosphorylation by native EnvZ. Cells will be cultured in Minimal Media supplemented with promoter inducer and the target metabolite. Upon reaching mid-exponential phase, fluorometric quantification of CFP and YFP will be conducted in 96-well plates to enable high-throughput screening of cancer biomarkers. Fluorescence thresholds will be defined to indicate positive detection.

The modular design allows rapid adaptation to new targets by swapping sensory domains. Future development of the screening assay includes reconstituting sensor cells into pellet formats for direct application to clinical samples, therefore streamlining diagnostic workflows. Our work highlights the potential of bacterial signalling systems as low-cost, scalable biosensors for early cancer detection, extending the utility of two-component systems beyond traditional environmental sensing.

B003

Low temperature drives the selection of robust and temperature-versatile microbial communities in granular activated carbon drinking-water biofilters.

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Abstract

Biofiltration is a sustainable method for drinking-water treatment that relies on microbial activity to remove contaminants. However, reduced winter temperatures may inhibit microbial metabolism and compromise performance. To evaluate the effect of temperature, an 11-month study was conducted using slow (3-hour EBCT) granular activated carbon (GAC) biofilters, feed the same influent water and operated at 10°C and 20°C. Performance was evaluated by Total organic carbon (TOC) and removal.

Surprisingly TOC removal showed minimal differences between temperatures, with only slight improvements at 20°C. Pathogen removal was unaffected by temperature. Illumina *16S rRNA* sequencing revealed that while the influent water community was highly similar, distinct microbial communities in the 10°C (GAC-10) and 20°C (GAC-20) biofilters formed indicating that temperature exerted selective pressure on community assembly.

A subsequent batch experiment was performed to assess the temperature response of the GAC-10 and GAC-20 microbial communities. Both were incubated at 6°C, 10°C, and 20°C, and their capacity to degrade organic carbon in identical influent water was measured after 8, 24, 48, and 72 hours. The GAC-10 community exhibited temperature flexibility, with no significant reduction in degradation across temperatures. In contrast, the GAC-20 community showed temperature sensitivity, degrading up to 90% less organic carbon at 6°C and 10°C compared to 20°C.

These results indicate that low temperatures do not necessarily limit biofiltration performance and can be used as a simple solution to select robust microbial communities, providing a mechanism for engineering biological performance.

B004

Genome-scale Metabolic Modelling Guided Design and Multi-omics Validation of Minimal Synthetic Microbial Communities for the Fermentation of Novel Water Kefir

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Abstract

Background

The concept of novel food fermentation has emerged as a response to converging factors, including the increasing demand for diverse and functional fermented foods, efforts to find novel solutions to global food shortages, and the shift towards more sustainable and plant-based diets. Water kefir (WK) is a traditional fermented beverage. This study used genome-scale modelling (GSM) to design minimal synthetic microbial communities (SynComs) for novel WK fermentations. These SynComs were subsequently validated through multi-omics analyses following fermentation with two food waste-based substrates from Ireland. The study aims to assess the performance of SynComs in terms of fermentation efficiency and to evaluate the safety and health-promoting potential of the resulting beverages.

Methods

Six SynComs were used for the fermentation. Shotgun metagenome sequencing was performed to identify the community composition after fermentation. Safety assessments, including mycotoxin screening and cytotoxicity evaluations, were also performed. Gut microbiome modulatory effects were evaluated using an ex vivo model of the distal colon. A comprehensive metabolomic analysis was performed to quantify non-volatile and volatile compounds.

Results

All SynComs successfully fermented the novel WK, lowering pH to ~3.5. Ethanol content remained within the non-alcoholic range (<0.3% ABV), and the sucrose content was 5g/l, resulting in non-alcoholic, low-sugar beverages. Shotgun metagenomics confirmed SynCom composition. No mycotoxins and cytotoxicity detected. Metabolomic analysis revealed the presence of favourable health-promoting compounds and volatiles, resulting in promising novel beverages.

Conclusion

This study demonstrates the successful application of GSM-guided SynComs to transform food waste-based substrates into novel, healthy, and sustainable fermented foods.

Session : Environmental, Applied & Industrial Microbiology Forum

B005

EPS-producing bacteria displaying the cell-separation phenomena in soil promote aggregation, preventing the leaching loss of nutrients

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Abstract

Leaching loss of nutrients from the agricultural field is a major concern in areas receiving excessive rainfall. The water-soluble nutrients are therefore lost due to leaching and runoff water. This leads to excessive dependence on chemical fertilizers. In this regard, the role of indigenous exopolysaccharide (EPS) producing bacteria in stabilizing the nutrient-holding capacity is far greater than conceived. The sub-Himalayan terai region, located in the Cwa zone (monsoon type with dry winter, Köppen's classification) in India, which receives heavy rainfall (>3000mm), is a suitable location to study the role of such bacteria. A culture-dependent analysis of EPS-producing bacteria showed their comparatively lower abundance throughout the year. The EPS-associated bacterial microenvironment on vermiculite particles under aerobic and anaerobic conditions was visualized using a Confocal Laser Scanning Microscope (CLSM). The cells of EPS producers were found distributed within the EPS matrix, pushing each other away, showing a higher organic matter secretion (EPS) per unit of bacterial cells. Since EPS has adhesive properties, the indigenous EPS-producing bacteria were amended to the soil, and the formation of macroaggregates was analyzed. Two in-house EPS-producing bacteria were used to monitor whether they can reduce the leaching loss of nutrients. *In vitro,* assays on nutrient-holding capacity by soil-column-flow-through revealed that these bacteria-treated soil retained more nutrients. To date, there have been no reports on the role of the genus Serratia in enhancing the nutrient-holding capacity. For the first time, we have demonstrated that utilizing soil-dwelling genera can enhance the formation of stable aggregates and mitigate nutrient loss.

B006

Bacterial inoculation can buffer the thermal fluctuations in fields, preventing low-temperature-induced membrane damage and electrolyte leakage from seedlings

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Abstract

Diurnal temperature fluctuations are ubiquitous in soil during the dry winter season in the Eastern Sub-Himalayan Terai region (Cwa zone, according to Köppen's classification). This difference in the evening and morning temperatures causes a huge loss in seed vigor, as seeds are in direct contact with the soil. Since the duration of soil temperature is not constant throughout the day, it was hypothesized that the duration of cold stress can have variable effects on the vigor of Solanaceous plants. When tested, it was found that there is a negative correlation between duration of cold stress and vigor up to a certain duration, and follows a one-step decay kinetics. This was because of the cold-associated membrane damage leading to the leakage of electrolytes. This was also verified by membrane-impermeable fluorescent dye uptake by the plant cells. To date, this stress existing in these regions has remained unnoticed. In this regard, biopriming the seeds with exopolysaccharide-producing microbes can be useful as the exopolysaccharide (EPS) can form a protective layer on the seeds. Treatment of the seed with these microbes reduced the electrolyte leakage, which improved the vigor under sub-optimal stress. Since EPS has a higher specific heat than soil, a field trial using a Randomized Block Design (RBD) was performed, and it was observed that the rhizosphere developed from bioprimed seeds displayed minimum fluctuations. This is the first report on using lesser-known microbiota of soil, like the *Phytobacter* and *Priestia sp.,* to buffer the soil thermal fluctuations.

B007

Horizontally acquired Lactoyl-glutathione lyase enables survival of Salmonella under salinity stress existing in the environment

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Abstract

Salmonella, a human pathogen, can colonize the members of the kingdom Plantae asymptomatically in agricultural fields and enter into the food chain. However, the understanding of the persistence of *Salmonella* in plants is largely unknown. As in an agro-ecosystem, various abiotic stress conditions are prevalent in the soil; we hypothesized whether methylglyoxal, one of the common metabolites accumulated in plants during both biotic and abiotic stress, plays a role in plant-Salmonella interaction. Methylglyoxal is one of the causes of free radical generation, which can have a detrimental effect on both animals and plants. We have investigated the interaction of *Salmonella*Typhimurium with the plant model *Arabidopsis thaliana* under salinity stress. We have observed that wild-type *Salmonella*Typhimurium can efficiently colonize Arabidopsis roots; however, mutant bacteria lacking the methylglyoxal (MG) detoxifying enzyme, lactoyl-glutathione lyase (Lgl), exhibit lower colonization in roots exclusively under salinity stress. We have further confirmed that the colonization defect is because of the immediate death of the mutant bacteria, which is mediated by plant-derived MG and not the de novo MG generated by these mutants. For the first time, we have shown MG as an anti-microbial metabolite of a plant. *Salmonella* serovar Typhi (*S.*Typhi, which causes typhoid in humans) lacking Lgl does not colonize the root; however, the ectopic expression of this enzyme can improve colonization, thus indicating the role of Lgl in root colonization under salinity stress. These findings contribute to our understanding of the trans-kingdom survival of Salmonella under various environmental stress conditions.

B008

Environmental predictors for the dynamics of the Harveyi and Splendidus clade pathogens in water, sediments, and mollusks.

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Abstract

Vibrio species of the Harveyi and Splendidus clades are the causative agents of vibriosis in aquatic organisms and are primarily responsible for seafood-related diseases in humans. We assessed the distribution of *V. parahaemolyticus*, *V. alginolyticus*, *V. harveyi/V. campbellii*, and *V. splendidus* in three Georgia (USA) *Crassostrea virginica* and *Mercenaria mercenaria* grounds. DNA from oysters, clams, water, and sediment was collected over the course of a year-long study and analyzed using qPCR. Species abundance in water, sediments, and mollusks was derived from the concentrations of species-specific *tlh*, VA1198230, *rpo*A, and *recA* genes, and profiled for water parameters and concentrations of the clade-specific virulence genes *tox*R, *lux*R, *srp, vhh*A, *vhh,* and *vhp*. *V. parahaemolyticus* was detected year-round in water, sediments, and mollusks in 61%, 44%, and 100% of the samples, respectively, followed by *V. splendidus* (67%, 17%, 17%), *V. alginolyticus* (17%, 0%, 22%), and *V. harveyi/V. campbellii* (19%, 33%, 0%). Water temperature ($r = 0.58–0.63$, $p \leq 0.05$), pH ($r = -0.46$), and dissolved oxygen ($r = -0.42$ to -0.56, $p \leq 0.05$) were reliable predictors for the abundance of the Harveyi and Splendidus clade pathogens. In water and sediments, the abundances of *V. harveyi/V. campbellii* and *V. parahaemolyticus* were highly correlated ($r = 0.80–0.99$, $p \leq 0.001$) with concentrations of most of the clade-specific virulence genes. The study revealed environmental predictors for monitoring the Harveyi and Splendidus clade pathogens and provided the first evidence for the presence of *V. harveyi/V. campbellii* in the Atlantic USA waters.

B009

Impact of Herbicides and Fungicides on Rhizobia and Other Soil Bacteria: Unveiling Neutral to Positive Dynamics Based on Soil and Active Substances

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Abstract

Pesticides play a key role in crop protection, yet their unintended effects on soil-beneficial microbes may undermine plant nutrition and broader ecosystem services such as nutrient cycling and soil fertility. While numerous studies explore microbial responses to pesticides, effects on specific groups like rhizobia remain underexplored, especially given the vast, shifting range of active substances. In this study, we assessed the direct effects of three fungicides and five herbicides on the diversity, abundance, and community composition of rhizobia and other soil bacteria. Pesticides were applied at recommended doses to three contrasting ecologically managed soils. Bacterial communities were profiled using Illumina paired-end amplicon sequencing, and total abundance was quantified via real-time PCR. The diversity and composition of rhizobial communities remained largely unaffected by pesticide treatments. In contrast, we observed a range of responses (positive, neutral, and negative) in the broader bacterial communities, depending on both the active ingredient and the soil type. Our findings indicate that while pesticides can markedly alter general bacterial diversity and abundance, rhizobia appear relatively resilient—suggesting either a lower sensitivity or a context-dependent response influenced by environmental factors. In particular, soil pH—both varying naturally among soils and altered indirectly by pesticide application—emerged as a key mediator of microbial community responses. These results highlight the importance of evaluating pesticide impacts not only on bulk microbial communities but also on functionally important groups such as rhizobia. Understanding soil-specific dynamics can help guide more targeted pesticide use, minimizing disruption to beneficial symbioses and supporting sustainable agroecosystems.

B010

Testing gelatine as a coating for the controlled release of calcium peroxide in cow rumen fluid

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Abstract

Our 2030 deadline to reduce methane emissions as part of our Global Methane Pledge commitment is fast-approaching. As cattle and other ruminant livestock account for 32% of our global methane emissions, it is no wonder they are the focus of a growing body of research into methane abatement strategies/techniques. The methane-inhibiting effects of the oxidiser calcium peroxide (CaO_2) has been substantiated both *in vitro* and *in vivo*. To meet the demands of pasture-based systems wherein cattle graze outside and there is limited contact for administration of a treatment, a slow-release feed additive solution has been proposed. As part of the Methane Abatement in Grazing Systems (MAGS) Project, the aim of this research is to identify and test suitable coating materials for the controlled release of calcium peroxide in cattle. Closed system bottle tests were first carried out to identify potential materials and investigate the impact of the treatment on methane production. Gelatine was the most promising candidate and was brought forward to a continuous dual flow fermenter trial during which the effect on oxidative reduction potential (ORP) and microbial community was observed. CaO_2 coated with gelatine successfully inhibited methane production in the batch tests and increased ORP in the fermenters. A decrease in archaea over time was seen in treated reactors. Future plans will involve a small-scale animal trial to investigate the impact of the treatment on digestibility and microbial community.

B011

SYNTHESIS OF BIOPLASTICS BY BACTERIAL STRAINS FROM ENVIRONMENTAL WASTEWATER: ISOLATION, SCREENING AND OPTIMIZATION STUDIES

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Abstract

Serious environmental menace is arising due to the accumulations of synthetic, non-biodegradable petroleum-derived plastics in the environment over the past decades. Globally, there is a pressing need for alternative biodegradable and environmentally friendly bioplastics. This study investigated the capabilities of environmental strains in the synthesis of polyhydroxybutyrate, known as bioplastics. Soil and wastewater samples of locust bean and cassava were collected from the locust bean and cassava processing factories at Ita-Oshin and Alabata, Federal University of Agriculture, Abeokuta. Enumeration and isolation of bacteria were carried out using standard microbiological techniques. Identification of bacteria strains was carried out using biochemical and molecular methods. Screening of bacteria for bioplastic synthesis was evaluated using the Sudan black assay and bioplastic was produced by potential strain. Culture conditions for optimized bioplastic synthesis (incubation time; 24h -120h, temperature; 30°C - 50°C; pH; 6 - 8 and inoculum volume; 1mL - 5mL) was ascertained varying one condition at a time. The predominant genera from cassava and locust bean wastewater were *Pseudomonas* and *Bacillus* while *Staphylococcus* and *Bacillus* genera were predominant in cassava and locust bean soil samples. Among the strains screened for the potential of bioplastic synthesis, *Bacillus cereus* from locust bean wastewater exhibited greater capabilities. The maximum bioplastic produced by *Bacillus cereus* was 3.46g/L under the optimized conditions at 35°C, 48 h, pH 7 using 3 mL of inoculum volume. This study revealed *Bacillus cereus* as an efficient bioplastics producer, suggesting it as a useful bio resource in commercial bioplastics production

B032

Enhanced Lactic Acid Biosynthesis by Mutagenized Lactic Acid Bacteria Isolated from Processed Milk

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Abstract

Lactic acid is a valuable weak organic acid widely used across food, pharmaceutical, and chemical industries. Traditionally produced through chemical synthesis, increasing demand for sustainable and eco-friendly methods has shifted attention toward microbial production. This study investigated the ability of multi-strain lactic acid bacteria isolated from processed liquid milk to synthesize lactic acid. The isolates were cultured on de Man Rogosa Sharpe (MRS) agar and identified based on morphological and biochemical characteristics. A promising isolate showing clear zones on MRS agar was identified as *Lactobacillus* sp. and subjected to mutagenesis using ethidium bromide (EB) and ultraviolet (UV) radiation. Thirteen mutants were obtained, and three were selected for lactic acid production studies using various substrates. High-Performance Liquid Chromatography (HPLC) analysis confirmed both mutants and parent strains as homo-fermentative lactic acid producers in cheese whey substrate. The parent strain yielded 2004.87 µg mL^{-1} of lactic acid, while UV and EB mutants produced 1457.67 µg mL^{-1} and 239.10 µg mL^{-1}, respectively. Optimal production occurred at 37 °C, pH 4.5, and 150 rpm after 16 hours of fermentation. Results indicate that mutagenesis did not enhance lactic acid yield; instead, metabolic shifts likely redirected carbon flow from lactic acid toward mixed-acid fermentation pathways, resulting in lower productivity.

B033

Microbial Enzyme–Mediated Synthesis of Silver Nanoparticles for Feather Waste Biodegradation

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Abstract

Feathers, a major by-product of the poultry and livestock industries, pose considerable environmental concerns due to their keratin-rich composition, which is highly resistant to microbial degradation. The accumulation of this recalcitrant waste contributes significantly to environmental pollution. In this study, an eco-friendly approach was developed for feather biodegradation through the microbial enzyme-mediated synthesis of silver nanoparticles (AgNPs). Microorganisms were isolated from poultry droppings and cow dung using Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA), respectively. Twelve distinct isolates were screened on skimmed milk agar for keratinase production. The isolate exhibiting the largest hydrolysis zone and highest keratinase activity was identified as *Curvularia eragrostidis* TDA4.2 and subsequently employed for AgNP synthesis. The resulting nanoparticles were characterized using UV–Visible (UV–Vis) and Fourier Transform Infrared (FT–IR) spectroscopy. UV–Vis analysis of AgNPs synthesized by *C. eragrostidis* TDA4.2, which recorded peak keratinase activity of 42.8 U/mL at 72 h, revealed a distinct absorption band between 420–435 nm, confirming nanoparticle formation. FT–IR spectra exhibited absorption bands within 1562.39–1554.68 cm^{-1}, attributed to C=C stretching vibrations of aromatic rings, indicating the involvement of biomolecular functional groups in Ag^{+} ion reduction. This study underscores a sustainable and biologically driven method for AgNP synthesis, highlighting the potential application of fungal keratinases in biotechnological processes for feather waste management and valorization.

B034

Antibiotic resistance genes in surface waters: distribution, drivers, and mitigation potential

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Abstract

Antimicrobial resistance (AMR) in surface waters represents a growing environmental and public health concern, with rivers and stormwater acting as key dissemination pathways for resistant bacteria and genes. We investigated factors driving AMR distribution patterns in river water and stormwater within an urban watershed. High-throughput qPCR and quantitative analyses revealed distinct AMR compositions between the two water types. At several river sites combined sewer overflow (CSO) discharges were associated with degraded water quality and pronounced clustering of resistance genes, while AMR gene distributions varied markedly across different land-use areas. In stormwater influenced by human activities, the presence of green infrastructure affected AMR profiles. Stormwater treated stormwater with green infrastructure exhibited AMR characteristics and water quality comparable to those of upstream river sections with minimal human disturbance, demonstrating green infrastructure's potential to mitigate AMR contamination. Overall, our findings highlight contrasting AMR signatures in riverine and stormwater systems, elucidate potential transmission pathways, and provide insights for managing urban water quality and controlling the environmental spread of AMR.

B035

Optimization of vitamin B12 fortification in fruit by-product–based matrices using propionic and lactic acid bacteria

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Abstract

An increasing number of people suffer from vitamin B12 deficiency, which is associated with numerous health consequences. Therefore, new approaches to enrich the human diet with B12 (e.g., through fermentation) appear necessary. The food industry generates millions of tons of by-products each year, including fruit pomace, which still possesses a rich composition and can be reused, e.g., in food production.

The aim of this study was to optimize B12 fortification in fruit pomace-based matrices using *P. freudenreichii* DSM20271 (PAB) monoculture and PAB + *Lactiplantibacillus plantarum* ATCC10241 (LAB) co-culture.

The cultures (120h, 30°C, pH≈6.5) were carried out in the matrices (sterile) containing fruit side-streams (one-, two-, and three-component matrices, without supplementation, DoE analysis). Strawberry, grape, and blue honeysuckle berry pomace were used. The following analyses were performed: B12 production (microbiological assay), bacterial growth (culture method), cobalt (ICP-MS) and riboflavin content (microbiological assay).

All tested matrices contained key precursors for B12 biosynthesis by PAB (cobalt, riboflavin). LAB showed significantly higher growth in the tested matrices compared to PAB. Experimental and statistical analyses indicated that the combination of 65% strawberry pomace and 35% grape pomace yielded the highest B12 biosynthesis in both the PAB and the PAB + LAB cultures. The PAB monoculture in the optimized matrix yielded 49.44±1.96 ng B12/g fresh matrix, while the co-culture produced 34.79±1.03 ng/g.

Fermentation of fruit side-streams can contribute to their use as valuable substrates in food production, following a zero-waste policy.

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B036

Non-Biofilm-Forming Mutants of *Staphylococcus aureus* Under Environmental Stress

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Abstract

Biofilm formation is a key survival strategy for *Staphylococcus aureus*, facilitating persistence under hostile conditions. However, the survival mechanisms of biofilm-deficient mutants remain poorly understood. This study investigated the ability of *S. aureus* mutants defective in surface attachment (ΔsrtA) and intercellular adhesion (ΔicaB, ΔicaC) to survive prolonged environmental stress compared with their respective wild-type strains (*S. aureus* NCTC 8178 and ATCC 15981). Using modified CDC semi-dry biofilm models, cell biomass and viability were assessed over 12 days under conditions of nutrient limitation and dehydration. Confocal laser scanning microscopy with LIVE/DEAD and molecular probe dyes was used to evaluate biofilm composition and cellular integrity. Despite their inability to form mature biofilms, all mutants demonstrated prolonged survival comparable to wild-type strains, with the highest biomass and cell recovery observed on day 12. The ΔsrtA mutant (1132) exhibited extensive cell damage under prolonged stress but maintained structural remnants enriched in proteins and extracellular DNA (eDNA). Notably, eDNA production appeared to compensate for the loss of surface-anchored proteins, facilitating limited adhesion and persistence. These findings suggest that *S. aureus* possesses multiple, biofilm-independent survival strategies, and that eDNA overproduction may represent a compensatory response to attachment or EPS deficiencies. This work highlights that biofilm formation is not the sole determinant of *S. aureus* persistence under desiccation and nutrient stress, emphasizing alternative mechanisms that may contribute to the long-term environmental survival of non-biofilm formers.

B037

Molecular analysis of extended-spectrum beta-lactamase-producing Escherichia coli among children and farm animals in Agogo, Ghana

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Abstract

Escherichia coli producing extended-spectrum β-lactamase (ESBL) enzymes are highly prevalent in Ghana, yet the one-health links driving their dissemination remain poorly understood. This study investigated ESBL-positive *E. coli* isolated from stool samples of Ghanaian children and farm animals (poultry and goats) using whole-genome sequencing (WGS). Among 117 isolates, 44 were from children without diarrhoea, 30 from children with diarrhoea, 35 from poultry, and eight from goats. Phylogenetic analysis based on multi-locus sequence typing (MLST) revealed limited co-colonization of children and poultry with identical clones. Common sequence types included ST617, ST38, ST10, and ST167. ESBL resistance was primarily driven by *blaCTX-M-15* genes, often found alongside other resistance determinants (*aph3-Ib*, *qnrS*, *sul2*, *tetA*, etc.). Enteroaggregative *E. coli* (EAEC) isolates were mainly associated with ST38, while some extraintestinal pathogenic *E. coli* (ExPEC) were also identified. Multiple plasmid incompatibility types, including IncFIA, IncFIB, IncFII, IncHI, and IncQ1 were detected and shared between human and poultry isolates.

Overall, the genomic evidence indicates shared sequence types, resistance genes, virulence factors, and mobile genetic elements among ESBL-producing *E. coli* from children and farm animals, suggesting potential interspecies transmission or common environmental sources. These findings highlight the importance of integrated, longitudinal One Health surveillance to better understand and mitigate the spread of antimicrobial resistance in Ghana and across West Africa.

B038

Production of Bacteriocins by Lactic Acid Bacteria Isolated from Nigerian Locally Fermented Foods for Food Preservation

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Abstract

Bacteriocins are natural antimicrobial peptides primarily produced by lactic acid bacteria (LAB). This study focused on isolating, characterizing, and optimizing bacteriocins from LAB recovered from traditionally fermented Nigerian foods to explore their potential in food preservation. A total of fifty LAB isolates were obtained from ten food samples: Kunun-zaki, Kunun-aya, Wara, Nono, and Daddawa. Isolation was performed by anaerobic culturing on de Man, Rogosa, and Sharpe (MRS) agar at 37°C for 72 hours, followed by morphological and biochemical characterization and confirmation through 16S rRNA gene sequencing. The LAB isolates obtained were *Lactococcus lactis* subsp. hordniae (86.61% identity), *Lactococcus garvieae* (96.61%), *Lactococcus lactis* strain CAU3142, and *Lactococcus garvieae* CAU9944. The bacteriocins produced by LAB isolates exhibited significant strain-dependent antibacterial activity against food spoilage bacteria namely; *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* Toxicological evaluation of the probiotics in albino rats at doses up to 5000 mg/kg revealed no mortality or significant adverse effects. When applied at 10% and 15% concentrations, these bacteriocins significantly suppressed microbial load, effectively extending shelf life compared to controls, which showed progressive spoilage. Additionally, bacteriocin treatments improved proximate nutritional profiles including protein, ash, and carbohydrate contents, and enhanced sensory properties and overall acceptability. The bacteriocins exhibit strong antimicrobial activity, safety, and the capacity to extend shelf life while improving the nutritional and sensory qualities of Masa and Akamu. These findings support their promising application as natural, sustainable food preservatives in Nigeria and beyond.

B039

Microbial production of lactic acid using agricultural wastes as a source of reducing sugars.

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Abstract

Lactic acid (LA) is an organic compound produced via fermentation by lactic acid bacteria (LAB) and has gained many industrial applications. One of the fundamental substrates and fuel source for lactic acid production is glucose. This study focused on the production of lactic acid using reducing sugar from Nigerian locally sourced agricultural wastes (corncob, sugarcane bagasse, plantain peduncle and groundnut shell). Bacteria were isolated from the agricultural wastes dumpsite and screened for lactic acid production using De Man Rogosa and Sharpe (MRS) agar. The agricultural wastes were pretreated using 1% NaOH and hydrolysed using cellulase produced by *Aspergillus niger.* The LAB were identifed based on their cultural, morphological, biochemical and molecular characteristics. The screened isolates were used for the production of lactic acid in an MRS medium containing the agricultural wastes hydrolysates. The LAB isolate with the best ability for lactic acid production was ascertained by spectrophotometry. Eleven (11) isolates were obtained from the agricultural wastes dumpsites. *Lactiplantibacillus plantarum* (accession number OM510300) from plantain peduncle dumpsites had the highest potential for lactic acid production (1.9558 g/L). The study revealed that Nigerian-locally sourced agricultural wastes can be developed as an alternative source of reducing sugar for lactic acid production, contributing significantly to the circular economy.

B040

Cryptic microbial hydrocarbon cycling in the ocean

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Abstract

Hydrocarbon production by photosynthetic eukaryotes and cyanobacteria is estimated to be approximately 500 times greater than that from fossil petroleum, highlighting their central role in hydrocarbon cycling in the ocean. Cyanobacteria are major producers of alkanes (C_{15}–C_{19}); however, eukaryotic microalgae, including haptophytes, dinoflagellates, and diatoms exceed them in both biomass and phylogenetic diversity, synthesizing a broader range of alkanes and alkenes (C_{15}–C_{38}). These compounds are not actively released but are instead liberated after phytoplankton cell death. Without hydrocarbon-degrading microbes, hydrocarbons derived from phytoplankton could accumulate to toxic levels in marine environments. Specialized microbial communities utilize these compounds as sole carbon and energy sources, maintaining a dynamic balance between hydrocarbon production and degradation. Understanding these cryptic interactions is essential for elucidating the mechanisms underlying hydrocarbon turnover in the ocean. To investigate these processes, seawater samples were collected across different depths and marine ecosystems in the North Atlantic and off the East Coast of England. Enrichment cultures were established using slowly released pentadecane as the carbon and energy source. Isolates obtained will be identified through 16S rRNA gene sequencing. Genome sequencing and substrate range experiments will assess their capacity to degrade different hydrocarbons, providing insights into whether they function as generalist or specialist hydrocarbon degraders, and potentially revealing novel taxa and degradation mechanisms. This study will be extended to include enrichments with major eukaryote-derived alkanes and alkenes, thereby enhancing our understanding of phytoplankton–microbe interactions and providing potential biotechnological applications, including bio-based hydrocarbon production and bioremediation strategies.

B041

Molecular Characterization and Optimization of Aspergillus niger for Enhanced Cellulase Production during Lignocellulosic Bioconversion

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Abstract

Molecular Characterization and Optimization of Aspergillus niger for Enhanced Cellulase Production during Lignocellulosic Bioconversion

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Background:

Lignocellulosic materials such as agricultural wastes are potential sources of renewable energy and other valuable products. The breakdown of these materials requires cellulase enzymes, which are commonly produced by fungi like Aspergillus niger. However, the enzyme yield from many fungal strains is often too low for industrial use. To improve production, there is a need to identify efficient fungal strains and optimize the fermentation process.

Aim:

This study aims to identify Aspergillus niger isolates and to optimize growth conditions that can enhance cellulase enzyme production for lignocellulosic biomass conversion.

Methods:

Fungal isolates will be collected from decomposing plant materials and identified using morphological and molecular methods. Submerged fermentation will be carried out using pretreated lignocellulosic substrates. The effects of pH, temperature, substrate concentration, and incubation time on cellulase production will be studied and optimized using statistical tools. Enzyme activity will be measured using the DNS method, and gene expression related to cellulase production will be analyzed using PCR.

Expected Results:

It is expected that process will lead to higher cellulase yield and that molecular analysis will confirm the identity of Aspergillus niger strains with strong cellulolytic ability.

Conclusion:

The results could contribute to the development of low-cost, sustainable processes for converting agricultural wastes into valuable bio-based products.

Keywords:

Aspergillus niger, cellulase, lignocellulosic biomass, enzyme optimization, fermentation

B042

Plasma-Enhanced Biodegradation of Polyurethane by Bacterial Co-culture

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Abstract

Polyurethane (PU), a polymer widely used in the production of foams, coatings, binders and elastomers, remains challenging in terms of its recyclability. While mechanical recycling requires extensive upstream sorting, chemical recycling is associated with environmental and health hazards. This study investigates the effect of plasma-induced surface modification of PU for enhanced biodegradation by bacterial co-culture. PU films were subjected to atmospheric plasma treatment at power levels of 60, 80, and 100% (max 750 W) after which they were subjected to biodegradation for 14 days using two bacterial co-cultures: *Streptomyces violaceoruber* in combination with *Rhodococcus ruber* (co-culture 1) and *Streptomyces violaceoruber* in combination with *Bacillus subtilis* (co-culture 2). Surface and structural changes were characterized by scanning electron microscopy (SEM), Fourier transformed infrared spectroscopy (FTIR), contact angle measurement, and differential scanning calorimetry (DSC). After 14 days of biodegradation, plasma-treated samples showed greater bacterial colonization, with visible cracks, and pits compared to untreated samples. FTIR analysis revealed changes in absorption peaks around 1726 cm^{-1} associated with carbonyl groups. Surface wettability increased significantly as the contact angle decreased from 94.3° to 46.2° and 45.8° in case of 80% and100% plasma-treated samples biodegraded by co-culture 1 and co-culture 2, respectively. DSC results showed a significant ($P \leq 0.05$) reduction in the glass transition temperature (T_g) for 100 % plasma-treated samples biodegraded by co-culture 1, compared to untreated samples; indicating degradation of amorphous PU regions. The results demonstrate that combining plasma treatment with microbial co-cultures promotes PU biodegradation, offering a strategy for sustainable PU waste management.

B043

Diversity analyses of microbial communities in Armanis gold-polymetallic mine and acid mine drainage: Bioremediation

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Abstract

Environmental pollution is among the most critical environmental concerns.Acid mine drainage (AMD) is considered to be one of the significant challenges that causes environmental damage worldwide. AMD is highly acidic and contains high levels of heavy metals,which threatens all living organisms. In this study, modern molecular approaches were used that allowed a more complete revealing the composition of microbial diversity in Armanis gold-polymetallic mine (Lori Region, Armenia).Data analysis identified 28phyla, 63classes, 129orders, 264families, and 738genera. *Proteobacteria* were found to be the most abundant phylum in all the sampling sites,with the highest abundance (95%) in all samples, followed by *Actinobacteria* and *Bacteroidetes*. It was shown that AMD samples contained autotrophic iron oxidizing bacteria belonging to genera *Leptospirillum*,*Acidithiobacillus*, *Sulfobacillus* as well as heterotrophic iron-reducing bacteria.The most abundant blue-green algae were referred to genera *Oscillatoria* and *Cyanobacteria.*Blue-green algae can be used to treat AMD by removing toxic heavy metals through a process called bioaccumulation.Thus, the study of diversity and dissemination of microorganisms in ore deposits of Armenia, as well as their activity in AMD generation, is very important and relevant from the point of view of their dangerous effects on the environment.On the other hand, they can be beneficially used for bioremediation of AMD and other industrial wastes.

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B044

Bathing Water as a Reservoir for Antibiotic-Resistant Escherichia coli: A Case Study from Newcastle, Northern Ireland

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Abstract

Antimicrobial resistance (AMR) is a growing global health crisis, it also presents distinct regional challenges driven by differences in local environment, population and antibiotic usage. In Northern Ireland data on AMR in bathing waters remains limited and current water quality legislation does not require AMR monitoring.

Surface water samples were collected from Newcastle, Co. Down (a designated bathing water), Omagh, Co. Tyrone (urban river system receiving agricultural inputs), and Craigavon, Co. Armagh (adjacent to Lough Neagh, a major recreational and drinking water source). Samples were filtered through 0.45µm cellulose-nitrate membranes and cultured on tryptone bile X-glucuronide agar with and without 4µg/mL cefotaxime (CTX) and CFU/100mL were determined. CTX-resistant *E. coli* isolates underwent antibiotic susceptibility testing (AST). DNA extracted from additional filters was used for microbial source tracking (MST) using human (HuBac) and ruminant (RuBac) markers.

E. coli concentrations positively correlated (<0.001) with rainfall 24h prior to sampling and temperature, indicating increased concentrations with wetter-warmer conditions. Similarly CTX-resistant *E. coli* concentrations also positively correlated (<0.001) with *E. coli* counts, rainfall and temperature and negatively with UV level. HuBac concentrations correlated with rainfall (<0.001), while RuBac correlated with rainfall and temperature (<0.001). highest CTX-resistant *E. coli* concentrations were recorded in Newcastle (log_{10} 2.2 CFU/100ml. AST revealed 52.6-100% resistance to ampicillin, amoxicillin-clavulanic acid, tetracycline, and norfloxacin with Newcastle and Omagh showing the greatest prevalence of multidrug resistance (MDR).

Our findings suggest that bathing waters can act as reservoirs for MDR bacteria, highlighting the need to incorporate AMR surveillance into water quality monitoring frameworks.

B045

Engineering myxobacterial super-predators to fight crop disease

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Abstract

The phylum Myxococcota encompasses an understudied group of Gram-negative bacteria: the myxobacteria. They are known to be producers of a wide range of bioactive metabolites that have therapeutic applications across the one-health spectrum. More recently, they are also gaining recognition as potential biocontrol agents. Traditional applications of chemical antifungals are commonplace in the agricultural industry to tackle crop diseases which can cause dramatic losses in yield and threaten food security. The use of biocontrol agents is a more sustainable alternative, and the soil-dwelling, spore-forming, predatory characteristics of myxobacteria make them ideal candidates. Septoria leaf blotch is a devastating disease of wheat caused by the fungal phytopathogen *Zymoseptoria tritici*. We have demonstrated the ability of various myxobacterial strains to predate on isolates of *Z. tritici* through *in vitro* predation assays, as well as the ability of some strains to impact *Z. tritici* biofilm formation. We have combined this with myxobacterial pangenomics in a genome-wide association study (GWAS) to identify genes of interest which have a significant correlation with predatory activity. Targeting these genes with gene editing strategies has the potential to generate engineered strains with enhanced predatory activity. Engineered strains may demonstrate increased potential as biocontrol agents in wheat plants, helping to sustainably target the wide-spread destructive effects of *Z. tritici*.

B046

Improving Recovery of Rare and Unculturable Fermentation Microbes Using the iChip.

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Abstract

BackgroundMany microorganisms in fermented food ecosystems remain uncultured using standard laboratory media, limiting our understanding of their ecological roles and functional potential. Water kefir contains a diverse microbiome in which sequencing frequently detects rare taxa that cannot be recovered by conventional cultivation. The iChip, an in-situ diffusion-based cultivation device, enables growth of these organisms by providing environmental conditions.

MethodWater kefir was used as the natural incubation environment for the iChip. Single cells from fresh fermentate were loaded into microchambers containing low-nutrient agar and sealed with semipermeable membranes. The iChip was then immersed back into active water kefir fermentation to allow growth under natural nutrient diffusion. In parallel, identical samples were cultured using standard laboratory media as a control. Recovered colonies were purified, re-streaked, and identified using full-length 16S rRNA Nanopore sequencing. Comparative analyses assessed recovery rate, taxonomic breadth, and overlap with sequencing-based community profiles.

ResultThe iChip yielded a higher number and greater diversity of isolates than all conventional media. Several taxa detected in community sequencing but absent from plate cultures were successfully recovered only through the iChip. Some isolates showed strict dependence on the fermentate environment, failing to grow when transferred to standard media. The iChip also improved the recovery of low-abundance and slow-growing taxa.

ConclusioniChip substantially enhances cultivation of rare and previously unculturable water kefir microorganisms by recreating natural environmental conditions. This approach bridges the gap between sequencing-based detection and cultivation, offering a powerful tool for studying complex fermentation microbiomes and expanding the repertoire of culturable taxa.

B047

Combined effects of biogenic zinc nanoparticles from Streptomyces sp. and palm shell mulching in improving water productivity and production in cucumbers cultivated in sandy soils in the United Arab Emirates

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Abstract

Mitigating water scarcity in agriculture necessitates new strategies to enhance water-use efficiency (WUE). This study examines the impact of four irrigation levels (100%, 75%, 50%, and 25% of crop evapotranspiration [ETc]) in conjunction with palm shell mulching (M), biogenic zinc nanoparticles (BznNPs) sourced from *Streptomyces* sp., or their combination (M + BznNPs) on water use efficiency (WUE), evapotranspiration, physicochemical properties, yield, and net profit of cucumber plants utilizing a deep drip irrigation system. Irrigation needs during the growth phase varied from 81.33 to 811 mm/day, thereafter decreasing to 15.44 to 3.1 mm/day at 25% ETc. The M and M + BznNPs treatments augmented fruit weight by 29-59%, whereas BznNPs improved yield by 31% at the lowest irrigation rate (IR 25%). The amalgamation of M and BznNPs treatments elevated carbohydrate, protein, and moisture content, dramatically diminished ETc, and enhanced WUE. The yield response factor was below the FAO (2002) guideline, indicating resilience to water stress. The energy savings from M + BznNPs treatments ranged from 31.55% to 72.25%. The application of IR at 25%, in conjunction with M and BznNPs, enhanced marketable yield, resulting in relative increases of 41-59% compared to the control group. The decrease in irrigation expenses augmented net benefits by 32%. The use of palm shell mulching and BznNPs, along with reduced irrigation (IR 25%), offers a sustainable approach to improve water-use efficiency, increase production, and optimize economic returns for cucumber cultivation in sandy soils in the United Arab Emirates.

B048

Improvement of yield, physiological traits, and oxidative stability of date palm (Phoenix dactylifera L.) trees subjected to salt stress with the application of biogenic selenium nanoparticles

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Abstract

Salinity presents a significant threat to agricultural output. This study investigated the application of biogenic selenium nanoparticles (BSeNPs) generated by *Streptomyces ferrugineus*, characterized by spherical morphology (41 nm diameter) and a surface charge of -25.3 mV, to alleviate salt stress in date palm trees. During two growing seasons (2022 and 2023), BSeNPs (0, 5, 10, 20, 40, and 80 mg/L) were administered as foliar sprays and soil drenches to evaluate their effects on growth, yield, and oxidative stability. Salinity stress decreased growth, chlorophyll concentration, relative water content (RWC), and ascorbic acid levels, while elevating catalase, ascorbate peroxidase, malondialdehyde, and hydrogen peroxide concentrations. The soil treatment of BSeNPs at 40 mg/L markedly enhanced chlorophyll content by 28.8-29.5%, relative water content by 33.3%, and ascorbic acid levels by 90.5%. It decreased proline by 48.8-51.7%, malondialdehyde (MDA) by 31.0-33.3%, and the activities of ascorbate peroxidase (APX) and catalase (CAT) by 32.1-40.0% and 16.7-25.0%, respectively, compared with untreated controls. SeNPs augmented the antioxidant defense mechanism, mitigated oxidative damage generated by reactive oxygen species, and diminished lipid peroxidation. This research provides the inaugural demonstration of the capability of BSeNPs sourced from actinobacteria to alleviate salinity-induced oxidative stress in date palm, while concurrently improving fruit yield and quality. BSeNPs provide a sustainable, eco-friendly alternative for safeguarding high-value crops, such as date palms, from the detrimental effects of salinity. This method not only protects agricultural yields under adverse environmental conditions but also promotes sustainable farming practices, underscoring the revolutionary impact of BSeNPs in contemporary agriculture.

B049

Microbial Source Tracking (MST) to Identify Human Faecal Pollution in Water

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Abstract

Faecal pollution threatens the ecological and public health of waterbodies, yet traditional faecal indicators cannot distinguish between human and animal sources. Globally, an estimated 1.8 billion people consume water contaminated with faecal material, contributing to disease outbreaks such as the 2023 *Cryptosporidium* incident in Devon, UK. Microbial Source Tracking (MST) provides a means to pinpoint the specific origins of contamination, enabling targeted remediation and risk reduction. We conducted a pilot study to establish the usefulness of a marker associated with *Bacteroides* spp. exclusive to the human gut and faeces, applying the USEPA-validated qPCR assay for quantification. Sampling was conducted at three locations in a 10 km stretch of an English River, up- and downstream of a wastewater treatment works serving over 200,000 people. Results showed a significant increase in the HF183 marker downstream of a wastewater treatment works ($p = 0.002$), demonstrating that treated effluent was the principal contributor of human faecal input. The study confirmed HF183 as a robust and rapidly detectable (same-day) molecular marker under UK conditions and highlighted the potential of MST to support targeted pollution mitigation, catchment management, and evidence-based regulation, contributing to improved river health and public trust in the water sector. Further work is underway to develop a complementary panel of animal-specific markers to enable comprehensive faecal source apportionment across catchments and further enhance the scientific basis for water quality management in the UK.

B050

Identification and mechanistic understanding of enzymes optimised for high pH detergent environments

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Abstract

The development of biological catalysts with functional adaptations for conditions such as alkalinity, is a topic of significant relevance, with a growing focus on improving their adaptability to challenging environments. Previous investigation into alkaline adapted enzymes provides some insight into critical features evolved to withstand this extreme environment, but with little focus on enzymes targeting carbohydrates. Extremophilic bacteria capable of degrading carbohydrates in high pH environments, such as salt lakes, represent a promising source of alkaline tolerant CAZymes for the furthering of this research area. This work focuses on mining the genome of Soap Lake bacteria as an origin of secreted carbohydrate active enzymes, functional under harsh alkaline conditions.

Ten alkaline native glycoside hydrolases from a Soap Lake bacteria have been heterologously expressed, purified and assessed for activity at pH 10. Among these, a glycoside hydrolase 26 mannanase, and a glycoside hydrolase family 16 glucanase, were structurally and functionally characterized, with a focus on the molecular features underpinning alkaline tolerance. Phylogenetic investigation into this alkaline mannanase has been utilised to evaluate the impact of surface charge on alkaline tolerance whilst the importance of carbohydrate binding module inclusion on alkaline catalysis has been investigated using the glycoside hydrolase family 16. Future efforts will involve protein engineering to confirm predicted structural features of alkaline tolerance with the later aim of engineering for enhanced performance of carbohydrate active enzymes at pH 10.

B051

Biofilms in constructed wetlands can support nutrient removal from urban surface water runoff.

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Abstract

Urbanisation has led to an increase in stormwater runoff. If unmanaged nutrients including phosphorus in stormwater runoff can leach into rivers and drive eutrophication in freshwater ecosystems. Natural microbial biofilms within constructed wetlands (CW) offer the potential for bioremediation of contaminants. This research aims to evaluate the bioremediation potential of natural biofilms in CW for phosphorus removal from storm water runoff and to characterise the microorganisms involved in these processes.

To evaluate the capability of biofilms to remove phosphorus, biofilms were developed under laboratory conditions using wetland water from Dronfield Lea Brook Valley, a flood meadow originally designed to reduce surface flooding. Biofilms were developed in soil leaching columns containing gravel as a substrate and left to develop for 14 days with a flow rate of 12ml/h. During the biofilm growth phase, biofilms were developed on the gravel using natural wetland water with and without P addition. At regular daily intervals, gravel and water were sampled to analyse the levels of phosphorus, determine phosphatase activity and obtain samples for subsequent DNA analysis. To characterise the microbial communities present in biofilms, amplicon sequencing of genetic markers (16S rRNA and ITS) was performed.

This research based on assessing the potential of CW biofilms for bioremediation of phosphorus in stormwater runoff, while laying the groundwork for future research exploring the capacity of biofilms to remove a broader range of contaminants, including persistent compounds such as per- and polyfluoroalkyl substances (PFAS).

B052

Listeria monocytogenes: Acid Adaptation capacity and Its Impact on Disinfection Efficacy

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Abstract

In 2023, European listeriosis fatality rate reached 19.7%, with 19 reported outbreaks. The main vehicle of infection remains ready-to-eat foods, which can be accidentally contaminated with *L. monocytogenes* during production[AD1] (EFSA Journal ,2024). Therefore, effective cleaning and disinfection of production environment is essential to prevent new listeriosis cases.

This study aimed 1) to assess the capacity of selected *L. monocytogenes* strains to adapt to acidic conditions, and 2) to compare the efficacy of acid and alkaline disinfectants on their formed biofilms. Ten *L. monocytogenes* strains originating from food processing environment were resuspended for 1 h in tryptic soy broth (TSB) adjusted to pH 5, and subsequently for 30 min at pH 3[AD2] . Afterwards, surviving strains were inoculated in microplates to form biofilm. Disinfection was performed by testing the efficacy of peracetic acid (PA, 10ppm), a widely used organic acid, and Top Active (TA, 2%), a commercial alkaline agent. Only five out of ten strains survived and were classified as acid adapted. Disinfection by TA completely eradicated the biofilms of the five acid-adapted strains. In contrast, treatment with PA was not effective.

These findings suggest that *L.monocytogenes* acid adaptation capacity is strain dependent and could reduce the efficacy of cleaning and disinfection procedures. The results of this study may contribute to a better understanding of the adaptive mechanism activated by L. monocytogenes to survive and persist in food processing environments.

B053

Dynamics of antimicrobial resistance genes in livestock, pasture and the environment under grassland fertilisation practices

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Abstract

The study investigated the influence of organic fertiliser on the abundance, diversity and presence of antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs) in livestock, pasture and associated environments. Organic fertilisers including manure, biosolids and slurry are used in agriculture to enrich farm soils. However, they may also introduce antimicrobial residues, antimicrobial resistant bacteria and associated resistance genes into the environment. ARGs and MGEs persist in agricultural ecosystems and may transfer between environmental and commensal bacteria, potentially impacting animal and human health. Understanding how grassland fertilisation practices affect ARG spread could identify farm management strategies that minimise AMR transmission through the food chain. We investigated the influence of different fertilisation practices on AMR bacterial selection and the transfer of ARGs and MGEs from soil to livestock and surrounding environment across two Scottish research farms. In a pilot study, biosolid application's impact on ARG and MGE diversity and abundance was assessed in pasture soils and animal faeces from two fields over multiple seasons. Resistome analysis detected various ARGs and MGEs across all samples with no comparative difference identified in gene diversity, or between samples collected from treated soil compared to control soil samples. There was no effect from biosolid treatment on gene abundance shown in exploratory analysis, principal component analysis or linear mixed models. In conclusion, preliminary findings suggest no evidence that biosolid application impacts ARG and MGE diversity or abundance in soil. Field-level replication could distinguish the impact of field characteristics from soil treatment on ARGs and MGEs.

B054

AmadoriZymes – Discovery and Characterisation of Novel Amadoriases for Cleaning Applications

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Abstract

Amadori compounds are intermediates in the Maillard reaction, formed when reducing sugars react with amino groups in proteins, peptides or free amino acids. Examples include fructosyl-lysine and fructosyl-valine, which are early precursors to advanced glycation end products (AGEs). While these compounds contribute to flavour and colour development in foods, their build-up in biological systems has been linked to ageing and certain chronic diseases. They also accumulate on textiles and cookware surfaces, causing yellowing and stubborn heat-modified residues that are difficult to remove with current cleaning formulations.

Many microorganisms encounter glycated substrates in the environments they inhabit, and several bacterial and fungal species are known to produce enzymes that act upon these compounds. Amadoriases, including fructosyl amine oxidases (FAOs), oxidise fructosyl-amines to generate the corresponding free amine, D-glucosone and hydrogen peroxide. These enzymes show promise as potential additions to detergent formulations, particularly because existing enzyme systems are ineffective against amadori products.

This project aims to identify and characterise new amadoriases from fungal and bacterial sources for use in fabric and home care applications. Proteomic analysis of selected strains will be used to identify candidate enzymes involved in fructosyl-amine turnover. These enzymes will then be expressed, purified and assessed against a range of fructosyl-amine substrates. Kinetic and structural studies will be carried out to determine the basis of substrate specificity and stability, informing later protein engineering. Small-scale laundry and dishwashing assays will test their performance under realistic cleaning conditions.

B055

Development of a Biofilm Drain Model and its Susceptibility to Treatment with Sodium Troclosene

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Abstract

Biofilms formed within hospital drains contain pathogenic bacteria and can pose a risk of infection to both patients and staff when water containing biofilm bacteria is splashed onto nearby surfaces and unintentionally transmitted. To reduce the risk of infections, the drain biofilm needs to be disrupted and disinfected. Previous studies have demonstrated that sodium troclosene (NaDCC) can effectively disrupt and kill bacteria within biofilms. NaDCC tablets release hypochlorous acid, a form of chlorine, when dissolved in water. Compared to bleach, NaDCC releases chlorine for longer time and works over a larger pH range. As such, it is widely used as a disinfectant for swimming pools and in the food industry. Therefore, the aim of this study was to develop a robust laboratory drain model to test the effectiveness of NaDCC against drain biofilms. A drain model using aspirator bottles and a peristaltic pump was established to mimic the sink drain and trap. Following the formation of a drain biofilm by *Pseudomonas fluorescens* and *Staphylococcus epidermidis*, two forms of NaDCC were assessed by confocal microscopy and CFU assays in their ability to kill and disrupt the biofilm structure. 1,000ppm of NaDCC resulted in a log-fold reduction of viable bacteria and no viable bacteria were detected following treatment with 4,000ppm when assessed by the CFU assay. Live/dead staining revealed a significant disruption of biofilm structure at 4,000ppm. NaDCC is a promising candidate for disinfection and removal of drain biofilms.

B056

Detection of airborne microbes and their derivatives using a novel ambient air sampling device.

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Abstract

Bioaerosols consist of airborne particles of biological origin, including bacteria, viruses, fungi, endotoxins and β-glucans. Bioaerosols can pose a range of risks such as disease transmission, food contamination, allergy and chronic health conditions. Hence there is an interest in measuring bioaerosols in environments where the occupational and public health risk is increased e.g. wastewater processing and healthcare. Multiple methods are available to measure bioaerosols, each with different capabilities and limitations. The aim of this study was to demonstrate the ability of a novel ambient air sampler (Apollo) to collect airborne microorganisms and their derivatives.

S. aureus, E. coli, L. innocua and *K. pneumoniae* and the bacteriophage MS2 were aerosolised within a test chamber. Air was sampled using the filtration based Apollo device. Filters were extracted and microbes quantified using culture based techniques and qPCR. Airborne endotoxin and β-glucans were sampled in residential properties for 7 days and quantified by recombinant factor C and Glutacell assays respectively.

Viable bacterial and viral particles from multiple species can be recovered and quantified from Apollo filters following short-duration sampling. Using qPCR techniques for quantification, the sampling time could be increased, resulting in higher quantification of bacteria from Apollo filters. Endotoxin and β-glucans could be detected and quantified from Apollo samples in all domestic properties.

The results of this study demonstrate the ability of Apollo to sample a range of microorganisms and their derivatives which are of interest in multiple industries. Apollo could provide a user-friendly solution for routine monitoring of bioaerosol exposure.

B057

Microbial Persistence and Transfer in Industrial Laundering Systems

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Abstract

Healthcare-associated infections (HAIs) remain a significant issue in clinical settings, where contaminated textiles and uniforms may contribute to pathogen persistence, cross-contamination, and biofilm development within laundry machines. Industrial laundries aim to proactively address these risks and optimise protocols for decontamination, yet the mechanisms of microbial survival and biofilm formation within these settings remain poorly understood. This study addresses these gaps by investigating how recycled water, treatment conditions, and textiles can influence contamination and biofilm formation.

Microbiome composition was assessed within multiple laundering system components to determine the persistence of biofilm-forming pathogens from healthcare textiles. Samples of lint, water, and textiles were collected after laundering cycles to assess biofilm formation, identify prevalent species, and determine routes of cross-contamination. Recycled water and tank samples without chemical or thermal treatment demonstrated evidence of biofilm formation. The microbial communities were profiled using 16S rRNA nanopore sequencing, highlighting the presence of opportunistic pathogens, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* spp., and other biofilm-forming species in recycled water modules as well as areas with limited chemical exposure. Furthermore, outlet water showed higher microbial loads than chemically pre-treated inlet water. These findings highlight the need for proactive disinfection strategies targeting both pathogens and biofilm reservoirs to maintain hygiene and operational efficiency within areas of industrial machines that are not subjected to chemicals or high temperatures. Future research will be conducted to address these areas and to develop strategies to minimise microbial persistence within laundering systems.

B058

Are pathogenic Vibrios emerging in the Teign estuary?

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Abstract

Vibrio species are Gram-negative bacteria found in coastal and estuarine environments. Due to climate change, sea surface temperatures (SSTs) are increasing, which increases the suitability for growth of warm water loving Vibrios in the environment. This has led to a poleward expansion of Vibrios into temperate areas, such as northern Europe. Outbreaks from human pathogenic Vibrios such as *V. parahaemolyticus* and *V. vulnificus* have already been reported in northern Europe. In the UK, knowledge of Vibrios in the environment is limited. The Teign estuary (Devon, England), is a shellfish hotspot with reported rising SSTs. This project involved the sampling of water, sediment and shellfish from the Teign estuary in 2024. Seasonal relationships were recorded between SST and Vibrio CFU/ml, with peaks in the summer months. Pathogenic species such as *V. parahaemolyticus* were isolated from all sample types and shown to be virulent in a *Galleria mellonella* insect model. Novel PCRs were developed to help determine between *V. diabolicus* and *V. alginolyticus* isolates, two closely related species. Additionally, the impact of sewage addition on the growth of *Vibrio* isolates from the Teign estuary were investigated.

B059

Off the Beaten Pathogen: Characterising the Host Interaction and Virulence Potential of Atypical *Salmonella* Uganda

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Abstract

Salmonella is one of the leading causes of diarrheal disease worldwide. *Salmonella* commonly resides within domestic, wild- and food-producing animals enabling infiltration into the food chain, resulting in salmonellosis through the consumption of contaminated products. *Salmonella* serovar Typhimurium is the most commonly isolated serovar causing foodborne outbreaks worldwide. However, factors such as travel, government policy and globalisation contribute to the increased emergence of atypical salmonellae, *Salmonella* Uganda being just one. Work thus far has sought to characterise this serovar and its unique phenotype. Ongoing studies have shown that this serovar tolerates extreme conditions, produces structurally sound biofilms at various temperatures, expresses swimming and swarming motilities and broader antimicrobial resistance profiles compared to Typhimurium. This serovar has also displayed distinct responses in infection models: *Galleria mellonella* and mice. In the *Galleria* model, infections with Uganda led to higher mortality rates than those infected with Typhimurium. Conversely, mice infected with Uganda exhibited weight maintenance or gain, greater survival rates, and an equal bacterial burden to mice infected with Typhimurium. Comparative genomics have also revealed the presence of unique genes essential for host infection and persistence as well as environmental tolerance. These findings point to Uganda as an underrecognised member of this genus highlighting the need for further research into this and other atypical serovars.

B060

Screening of foodborne pathogens under the combined effect of high-frequency ultrasound and green tea extract

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Abstract

Increasing consumer demand for minimally processed foods has encouraged the development of non-thermal disinfection methods as alternatives to conventional heat-based treatments. This study evaluated the antimicrobial potential of high-frequency ultrasound (HFUS, 760 kHz, 30 W) applied alone and in combination with green tea extract (GTE, 5% w/v). Seven foodborne bacterial strains, comprising both Gram-negative and Gram-positive species, were selected. Sonochemical activity was characterised to optimise frequency, exposure time, and sample position for maximum reactive species generation using a custom-designed ultrasonic set-up.

Bacterial suspensions were subjected to individual and combined treatments, and viability was assessed using plate counting and flow cytometry to evaluate membrane integrity. Scanning electron microscopy (SEM) images were used to visualise morphological changes. Ultrasound alone exhibited greater efficacy against Gram-negative bacteria by inducing mechanical damage on cell surfaces, whereas GTE was more effective against Gram-positive strains. The combination of ultrasound and GTE resulted in a greater reduction in viable counts than either treatment alone and a marked increase in sublethal injury levels across both bacterial types. Rod-shaped cells exhibited more pronounced structural damage than coccal forms under ultrasound. Catalase activity was assessed through foam formation, with the combined treatment showing higher foam production, indicating increased oxidative damage on bacterial cells.

These findings demonstrate that coupling HFUS with natural plant antimicrobials enhances bacterial susceptibility through complementary mechanisms attributed to cavitation-induced membrane disruption and polyphenol-mediated oxidative stress. The combined treatment represents a promising, sustainable, and non-thermal strategy for the effective control of foodborne pathogens in food safety applications.

B061

Wastewater Surveillance for COVID-19 Outbreaks at a Ward Level: Emerging Insights from Bengaluru, India

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Abstract

COVID-19 pandemic has highlighted the need for evidence-based, effective methods to allocate limited public health resources, especially in resource-constrained settings. Wastewater surveillance offers such a cost-effective approach to rapidly identify COVID-19 hotspots. This study aimed to develop a standardized protocol for sampling and testing of SARS-CoV-2 causing COVID-19 in mixed and untreated wastewater in Bangalore City, India, and to evaluate whether sewage levels of viral RNA correlate with community infection rates. We conducted longitudinal monitoring in 45 densely populated wards of Bengaluru, India covering one-third of the population. Wastewater samples were collected twice weekly from storm drains and periodically from sewage treatment plants. Samples underwent heat inactivation, filtration, polyethylene glycol precipitation, RNA extraction, and reverse transcription polymerase chain reaction analysis. We developed a real-time dashboard to track viral loads and trends, integrating sewage data with community testing and positivity rates. Wastewater surveillance detected viral RNA trends that corresponded with community infection rates. Increases in viral loads in sewage preceded spikes in reported cases by approximately one week, demonstrating the potential of sewage surveillance as an early warning system. Wastewater surveillance is a valuable tool for early detection of coronavirus disease 2019 outbreaks at the community level and can inform targeted public health interventions. Implementing such surveillance in resource-limited settings can enhance pandemic preparedness and response. Further research is needed to address data gaps and optimize methodologies for reliable, actionable insights.

B062

Entomopathogen control within the insects for food and feed industry utilising the predatory bacterium *Myxococcus xanthus*

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Abstract

Current UN predictions estimate the global population will reach 8.5 billion by 2030 and 9.7 billion by 2050. This continuing growth puts pressure on already unsustainable agricultural systems with increasing requirements for high-quality protein. 'Insects for food and feed' is a promising alternative protein stream, which also helps to tackle the need for more adaptable, sustainable and less environmentally damaging forms of agriculture. Insect rearing facilities are intensive - typically see high stocking densities, humidity and temperature, compounded by a lack of legislation as to the mandatory husbandry of insects. Such conditions promote pathogen outbreaks, resulting in potential economic losses and welfare issues.

Myxobacteria are soil-dwelling social bacteria which move and feed cooperatively in predatory groups. Their wide prey range and predatory characteristics mark myxobacteria as an exciting and promising candidates for new biocontrol treatments. We have been investigating the therapeutic effects of the myxobacterium *Myxococcus xanthus* when applied to desert locusts (*Schistocerca gregaria*, Orthoptera) infected with the entomopathogenic fungus *Metarhizium robertsii.* Survival studies showed that *M. xanthus* can protect *S. gregaria* from *M. robertsii* infection. We have also investigated the locust gut microbiome, which is diverse and variable, finding that *M. xanthus* can reach the insect gut without triggering systemic dysbiosis. Our current research is exploring the effects of myxobacterial treatments on the insect microbiome when challenged with an entomopathogen, and the potential benefits of the use of *Myxococcus xanthus* as a probiotic to reduce microbial loads within the insect gut prior to harvest.

B063

Characterization of bacteriocin and biofilm-producing E. faecalis isolates from RTE foods and water

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Abstract

In the food industry, *Enterococcus* species play dual role, functioning as beneficial probiotics in cheese fermentation while acting as spoilage organisms in meat. Recognized as indicators of fecal contamination, they have been implicated in foodborne and nosocomial infections. Certain strains produce bacteriocins inhibiting pathogens such as *Listeria monocytogenes*, thereby enhancing food safety; however, their full potential in the food sector remains underexplored.

This study investigated bacteriocin production, biofilm formation, and antimicrobial resistance (AMR) in *Enterococcus faecalis* isolates from ready-to-eat (RTE) foods and water samples in Accra, Ghana, to evaluate their implications for food safety. Fourteen isolates were assessed for biofilm formation, bacteriocin activity, AMR, and whole-genome sequencing to identify genes of interest.

Although 13 of the 14 isolates carried biofilm-associated genes, six lacked phenotypic biofilm expression. Five isolates were multidrug resistant, exhibiting resistance to erythromycin (5/14) and linezolid (3/14). Two isolates were resistant to chloramphenicol, penicillin, and ciprofloxacin, while four were resistant to tetracycline and vancomycin. Resistance genes *lsa(A)*, *cat*, *ermB*, *tet(M)*, and *tet(C)* were detected alongside biofilm genes (*ebpA*, *ebpB*, *ebpC*) and plasmid replicons (*rep9a*, *repA(pTW9)*, *rep9b*, *rep22*, *rep32*, *rep9t*, *repUS43*, *col3M*). Clinically relevant sequence types ST16, ST79, ST126, ST81, ST242, and ST376 were identified.

Findings suggest that biofilm formation is influenced by environmental regulation rather than gene presence alone. The coexistence of virulence and resistance traits in foodborne *E. faecalis* underscores their significance as emerging threats to food safety and public health, emphasizing the need for genomic and phenotypic monitoring in the food industry.

B064

Development of an ACE2-Based Electrochemical Biosensor for Airborne SARS-CoV-2 Detection in Care Home Environments

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Abstract

Rapid detection of airborne viral pathogens is essential for infection control, particularly in high-risk environments such as care homes. This study reports the development of an electrochemical biosensor for near real-time detection of SARS-CoV-2 in air samples. The sensor employs laser-induced graphene (LIG) electrodes functionalised with the angiotensin-converting enzyme 2 (ACE2) receptor, enabling specific binding to the viral spike (S1) protein. LIG electrodes were fabricated on polyimide films via CO_2 laser engraving and characterised using scanning electron microscopy and Raman spectroscopy, confirming successful formation of high-quality graphene. Surface modification with cross-linkers (phenylboronic acid, PBA; and 1-pyrenebutyric acid N-hydroxysuccinimide ester, PBASE) facilitated stable ACE2 immobilisation. The unique pi-densities of LIG surface allows the bio-functionalization to happen via pi-stacking interactions, eliminating the need for conventional self-assembled monolayer (SAM) formation, which makes the biosensor formation simpler and faster. Electrochemical analyses, including square wave voltammetry (SWV), demonstrated concentration-dependent decreases in peak current upon exposure to recombinant SARS-CoV-2 S1 protein. Statistical validation (one-way ANOVA, Dunnett's test, ****$p < 0.0001$) confirmed significant responses at ≥0.1 ng/mL. Integration of this biosensor with portable air samplers is underway to enable on-site detection of airborne SARS-CoV-2. These findings highlight the potential of ACE2-functionalised LIG sensors as field-deployable platforms for environmental viral surveillance in healthcare settings.

B065

Narrow spectrum antibiotics for the prevention and treatment of soft rot plant disease

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Abstract

Worldwide losses to plant diseases are conservatively estimated at approximately US $150 bn, of which about one third are attributable to bacterial infections. Soft rot plant disease is caused through the infection of plants by bacteria such as Pectobacterium spp. and can occur in a range of economically important crops such as potatoes.

In many cases, good sources of resistance for breeding are not available and the chemicals used to prevent spoilage are increasingly deemed environmentally unacceptable. The use of broad-spectrum antibiotics in the food chain is also undesirable as this can lead to the selection of antibiotic-resistant strains of bacteria that may ultimately cause infections in humans. Bacteriocins are potent naturally produced protein antibiotics that have a narrow-killing spectrum and could be deployed to target a specific pathogen while leaving the wider plant and soil associated microbiomes intact.

Our current work focusses on the identification, production and testing of novel bacteriocins targeting Pectobacterium spp. We are using a range of bioinformatic, genomic and biochemical tools to identify and determine the mechanism of action of Pectobacterium targeting bacteriocins. We have also tested the efficacy of bacteriocins in soft rot plant disease models using an image-based potato tissue infection assay.

B066

Feedstock-Driven Selection Constrains *Methanosarcina* Bioaugmentation in Anaerobic Digesters

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Abstract

Previous observations at Advanced Anaerobic Digester facilities revealed that *Methanosarcin*a-dominated digesters consistently outperformed *Methanosaeta*-dominated systems in biogas production under similar operating conditions. This study investigated whether bioaugmentation with *Methanosarcina*-enriched inoculum could enhance methane production at lower-performing sites. Microcosms were established using five *Methanosarcina:Methanosaeta* inoculum ratios (100:0, 75:25, 50:50, 25:75, 0:100), all fed exclusively with feedstock from a lower-performing site. Microbial community structure was characterised through 16S rRNA gene sequencing, with functional profiles predicted using PICRUSt2. A *Methanosarcina*-specific qPCR assay was developed and validated to enable quantitative monitoring of target populations across treatments. Despite differing initial methanogen ratios, mixed inocula converged toward *Methanosaeta*-like functional profiles, indicating strong feedstock-driven selection. Statistical analyses confirmed weak but significant community differentiation (ANOSIM $R = 0.251$, $p = 0.044$; PERMANOVA $R^2 = 0.483$, $p = 0.027$), with SIMPER analysis identifying ABC transporters as primary contributors to group dissimilarity, though effect sizes remained low, suggesting high functional redundancy. Only pure *Methanosarcina* inoculum maintained distinct metabolic potential. qPCR confirmed *Methanosarcina* establishment across all mixed treatments, yet functional profiles still converged toward *Methanosaeta*-dominated communities despite comparable methanogen abundance. Feedstock composition overrides taxonomic differences and constrains bioaugmentation efficacy in anaerobic digestion systems. Enhancing biogas production at *Methanosaeta*-dominated sites likely requires process optimisation strategies beyond inoculum manipulation alone.

B067

Comparison of direct and indirect cold plasma treatment of common uropathogens

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Abstract

Non thermal or 'cold' atmospheric plasma (CAP), a wholly or partially ionized gas generated at or near ambient temperatures and pressure, by electrical breakdown of a carrier gas, is a novel antimicrobial strategy based on reactive oxygen and nitrogen species, with demonstrated efficacy against planktonic and biofilm bacteria. The delivery of CAP to the site of infection is an important therapeutic consideration. Treatment can be by direct exposure of the target to the CAP jet plume, or by indirect exposure, involving exposure (plasma activation) of a liquid to a CAP discharge, imbuing the liquid with CAP's antimicrobial properties via translocation of longer-lived reactive species into the bulk liquid. This study compares the antimicrobial activity of direct CAP exposure and plasma-activated water (PAW) treatment against type strains and clinical isolates of common uropathogens: *E. coli*, *K. pneumoniae*, *E. faecalis*, *P. mirabilis*, *S. saprophyticus*, and *P. aeruginosa*, in planktonic and biofilm states. Planktonic cultures of clinical isolates proved much more tolerant of CAP treatment than the type strains, resisting eradication after up to 5 min direct exposure and 30 min PAW treatment. Chemical scavengers showed hydrogen peroxide was critical for the biocidal activity of indirect plasma. Direct CAP exposure led to rapid bactericidal activity, eradicating biofilms within 60 s and most planktonic cells within 5 min, however delivery to the urinary tract would be challenging. By contrast, PAW demonstrated promising antimicrobial activity and could be more readily delivered, eg as catheter lock or bladder irrigation solution, for control of urinary tract infections

B068

Cooperation or Conflict: How do Environmental Changes Impact Vibrio Dormancy Resuscitation and Competition?

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Abstract

Vibrios are a genus of bacteria present in brackish water at temperatures above 18ºC. Species within this genus can cause diseases such as gastroenteritis and necrotising fasciitis, sometimes leading to sepsis. Vibrios can enter a state of dormancy due to stress or suboptimal growth conditions, where they are metabolically active but have a low growth rate, so they are unculturable with traditional plating techniques. Dormancy reversal or resuscitation is achieved by restoring optimal growth conditions or adding exogenous substances. A metabolite called lactate has demonstrated *Vibrio* resuscitation in controlled conditions, but its environmental applicability was unknown. This study shows that lactate can resuscitate dormant Vibrios from environmental samples collected from the River Teign, causing a shift in species resuscitated rather than an increase in total abundance. This study finds lactate reservoirs in shellfish samples collected from the river for potential lactate-mediated resuscitation. To understand the *Vibrio* species shifts, this study also investigates the *Vibrio* interspecies dynamics in different environmental conditions and determines whether dormancy has a role in these dynamics. Understanding how dormancy resuscitation occurs and influences species proportions is essential for effective disease surveillance to minimise outbreaks of *Vibrio* infections.

B069

Deciphering the molecular mechanisms of Glyphosate metabolism by Sinomonas atrocyanea ATCC13752

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Abstract

Glyphosate (N-(phosphonomethyl)-glycine), a broad spectrum organophosphonate herbicide, is the most widely used herbicide globally. Despite its dominance in modern weed management, its widespread usage has been linked with adverse ecological and health complications. While biodegradation is a more sustainable and effective long term remediation strategy to mitigate the ecotoxicological consequences, surprisingly, fundamental questions still remain to be answered about its metabolism by microorganisms.

Sinomonas atrocyanea ATCC13752, is a Gram positive bacterium deposited in culture collection prior to the invention of glyphosate. Pipke and Amrhein (1988) reported its ability to completely degrade glyphosate, producing AMPA (Aminomethyl phosphonic acid) as its primary metabolite with the subsequent release of phosphate and CO_2. We aim to apply a combination of molecular techniques in order to understand glyphosate metabolism in *S. atrocyanea* and its evolutionary root.

Although *S.atrocyanea* grew on glyphosate and KH_2PO_4 as sole phosphorus sources, consistent with the work of Pipke and Amrhein (1988) it unexpectedly, showed no growth on AMPA. Similarly a range of other organophosphonates provided as sole source of phosphorus also failed to support growth.

Whilst the genome of *S. atrocyanea* ATCC13752 is available, there is little to help us understand the genes involved in glyphosate transport and metabolism with no known phosphonate metabolizing genes annotated or indeed alluded to after bioinformatic investigation. A proteomic workflow has been created that will allow us, for the first time to understand more about not only glyphosate degradation, but also phosphorus metabolism more widely, in *S. atrocyanea* ATCC13752.

B070

Predatory myxobacteria as biocontrol agents against fungal entomopathogens in edible insect farming.

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Abstract

Edible insect farming is an emerging sustainable protein production technique; however, it faces challenges posed by recurrent disease outbreaks caused by microbial entomopathogens. Conventional chemical and antibiotic-based crop protection tactics have been used for a long time, but they are generally ineffective or pose food safety concerns; therefore, they should not be used for insect farming. Myxobacteria are a diverse group of soil-dwelling, predatory microorganisms that predate bacteria and fungi while producing a wide range of bioactive secondary metabolites, making them interesting candidates for biological control. This study investigates the potential of predatory myxobacteria to suppress fungal pathogens affecting the desert locust (*Schistocerca gregaria,* Orthoptera). The study involves isolating and characterising fungal pathogens associated with desert locusts, testing their pathogenicity, isolating novel myxobacterial strains, assessing their predatory activity against entomopathogenic fungi in vitro, and validating their biocontrol efficacy in vivo within controlled insect farming systems. Two fungal species, *Aspergillus flavus* and *Aspergillus tamarii,* were isolated from naturally deceased desert locusts and showed pathogenicity when reintroduced to healthy locust populations, resulting in much higher mortality than untreated controls. Furthermore, treatment with a consortium of myxobacteria significantly reduced the proliferation and pathogenicity of both fungal species. This antagonistic action was demonstrated both in vitro and in vivo, showing that myxobacterial consortia can be efficient biocontrol agents against fungal entomopathogens. Future experiments will determine the mechanisms of protection of these myxobacteria. The findings of this research aim to advance ecological, safe, and scalable biocontrol solutions for edible insect farming.

B071

Development of a semi-dry wound biofilm model for quantifying interkingdom biofilm dynamics

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Abstract

Introduction: Diabetic foot ulcers (DFUs) are chronic wounds characterised by polymicrobial biofilms that impair healing. Among these, *Staphylococcus aureus* (Sa), *Candida albicans* (Ca) and *Pseudomonas aeruginosa* (Pa) are associated with poor outcomes, yet their interkingdom interactions aren't well captured in standard laboratory models.

Aims: To identify dominant microbial species in DFUs and develop a semi-dry wound biofilm model to investigate clinically relevant microbial interactions.

Methods: DNA was extracted from DFU wound swabs and qPCR performed for Sa, Pa and Ca. A sodium alginate hydrogel (SAH) substrate was prepared, and mono-, dual- and triadic-biofilms were generated on wound dressing membranes placed onto the SAH surface. Biofilm biomass was quantified by CFU enumeration.

Results: Sa was detected in ~95% of swabs (mean $\log_{10}$ CFE ≈ 4.0), with 77% exceeding $\log_{10}$ 3, a threshold considered clinically significant. Ca was detected in ~85% of swabs (mean $\log_{10}$ CFE ≈ 2.9), with 42% exceeding $\log_{10}$ 3. Pa analysis is ongoing. In the SAH model, Ca colonisation was higher on the dressing than the gel (5.5×10^6 vs 1.6×10^6 CFU/mL), while Sa biomass was consistent across surfaces (2.6×10^7-2.9×10^7 CFU/mL). In dual-species biofilms, Sa growth increased (up to 1.2×10^8 CFU/mL) and Ca viability decreased, demonstrating potential cross-kingdom interactions. Tri-species tests are ongoing

Conclusions: We've shown that paradigm biofilms formers Sa, Pa and Ca are found in DFU infections. This semi-dry model reflects relevant biofilm behaviour and interkingdom dynamics. Its reproducibility and physiological relevance make it a practical platform for screening therapeutics

B072

Effect of Strain Selection, Inoculum Density, Crystal Violet Concentration and Solubilization Duration in the Crystal Violet (CV) Assay for Standardizing Biofilm Quantification

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Abstract

The crystal violet assay is a widely used method for quantifying biofilm formation in microbiological studies. However, the accuracy and reproducibility of this assay can be influenced by several experimental variables. In this study, we systematically evaluated the effects of bacterial strain variation, inoculum density, crystal violet (CV) concentration, and duration of solubilization on biofilm quantification. Bacterial strains with strong, intermediate and weak biofilm-forming capabilities were assessed under varying parameters including the initial cell densities. The effects of crystal violet concentration and the duration time of solubilization were analysed to understand maximum staining efficiency and to minimize variability. Our results demonstrate that each variable significantly impacted the biofilm biomass measured, with the most sensitive variables affecting assay conditions being strains and CV concentrations. These findings highlight the importance of standardizing CV-based biofilm assay parameters and provide practical recommendations for improving the reproducibility and comparability of biofilm quantification.

# Authors contributed equally

B073

Copper and zinc from slurry applications shape grassland microbial community structure

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Abstract

Soil microbes are crucial in maintaining soil health and enhancing agronomic productivity. This study investigates long-term cow and pig slurry applications on soil microbial community structures, soil chemistry, and herbage yield in a ~50-year Long-Term Slurry (LTS) experiment on a permanent grassland located in County Down, Northern Ireland. Illumina and PacBio rRNA sequencing (analysing 16S and ITS genera) were used to investigate soil samples from the experiment's treatments to depths of 15cm. Slurry type and application rate were found to alter the relative soil microbial community. In comparison to the non-amended control treatment and inorganic fertiliser treatment, higher slurry rates had the greatest log2 fold change of relative microbial abundance, alongside increasing soil pH and total carbon. Soil pH, copper and zinc concentrations were found to be significant key drivers of soil microbial community shifts (permutation test, $P<0.001$). More specifically, copper primarily influenced 16S and zinc was the greatest influence on ITS genera. CAP analysis revealed that relative abundance changes of 16S genera diverged depending on slurry type and rate, with ITS genera only distinguished by rates of slurry.

B074

Presence of *Campylobacter* spp. in red deer (*Cervus elaphus*) faeces collected from ten Scottish deer farms

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Abstract

Objective: *Campylobacter* spp*.* are present as gut commensals in many animal species. Human disease is most commonly caused by *Campylobacter jejuni* and *Campylobacter coli* with undercooked poultry as the most common source of infection. However, livestock have also been identified an important reservoir for human infection in the UK. Carriage of Campylobacter by UK farmed deer (*Cervus elaphus*) is understudied and associated risks to public health are unknown. Here we investigate the presence of *Campylobacter* spp*.* in farmed deer faeces from deer-only and mixed-enterprise farms and the potential role of farmed deer as a reservoir for *Campylobacter* spp.

Methods: Deer faecal samples (n=323) were collected from 10 commercial deer farms in Scotland in 2022. On four farms, deer co-grazed with either cattle, sheep or both. Swabbed samples were enriched in Exeter broth for 2 days at 37^{o}C, plated onto CCDA agar and incubated under microaerophilic conditions at 37^{o}C for 72h. DNA was extracted from 40 isolates using QIAGEN Blood and Tissue kit and Illumina short read sequencing was performed.

Results: *Campylobacter* spp. was isolated from 193 samples (60%) and from all 10 farms. 90% of isolates were *Campylobacter hyointestinalis* and 10% were *C. jejuni*. Sequencing data was also interrogated to investigate presence of AMR genes.

Conclusions: A high incidence of *C. hyointestinalis* was detected in deer faeces collected. Phylogenetic analysis, alongside comparison of *Campylobacter* isolates carried by cattle and sheep may inform about the aetiology of strains and risk of transmission.

B075

Quorum Sensing and Biofilm Regulation Mediated by pctA and tlpQ Genes in Meat-Derived Pseudomonas Isolates

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Abstract

Red meat, one of the most consumed protein sources, provides a favorable environment for microbial spoilage. Among the microorganisms responsible for aerobic spoilage under refrigerated conditions, *Pseudomonas* species are predominant due to their ability to form robust biofilms and remain metabolically active at low temperatures. Biofilm formation is closely associated with quorum sensing (QS), a bacterial communication system that regulates gene expression based on cell density. QS involves signaling molecules such as acyl-homoserine lactones (AHLs) in Gram-negative bacteria, oligopeptides in Gram-positives, and the universal signal molecule autoinducer-2 (AI-2), derived from 4,5-dihydroxy-2,3-pentanedione (DPD). Although *Pseudomonas aeruginosa* does not produce AI-2, it can sense environmental AI-2 through the recently identified chemoreceptors PctA and TlpQ, which belong to a distinct receptor family. Literature on QS systems in meat-spoilage *Pseudomonas* species remains scarce. In this study, four *Pseudomonas* isolates—three local isolates (YK107, YB91, YK50) and the type strain *P. fragi* ATCC 4973—were selected based on their strong biofilm formation and AI-2 production capacities. The *pctA* and *tlpQ* genes were targeted for deletion by amplifying their upstream and downstream regions via PCR, followed by fusion using overlap extension PCR and cloning into the pK18sB vector. Homologous recombination will generate mutant strains, which will be evaluated for AI-2 production using *Vibrio campbellii* ATCC BAA-1117 as a biosensor. Biofilm formation and motility assays will elucidate the roles of *pctA* and *tlpQ* in QS and biofilm regulation.

B076

Perspectives on Biofilm Management: Results from the Biofilm Alliance Implementation Phase Survey

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Abstract

Microbial biofilms significantly impact health, food safety, industrial processes and the economy at large, costing an estimated US$5 trillion annually sectors-wide. The Biofilm Alliance, an Innovate UK-funded Regulatory Science network, aims to bridge the gap between advances in biofilm research and evidence-based regulatory frameworks by stimulating collaboration among academia, industry, regulators, and standards bodies.

Here, we present the findings from the Biofilm Alliance Discovery and Implementation Phase Surveys to gather international perspectives on biofilm challenges and current practices, regulatory environments and barriers, innovations, capacity building, and collaborative needs in the food, water, built environment and industrial processes sectors. The surveys received anonymous responses from diverse stakeholders, including academics, industry, antibiofilm technology developers, and testing/measurement providers, with an equal split in responses from the four sectors.

The Discovery Phase Survey respondents indicated widespread application of biocides to attempt to control biofilms, despite some uncertainty over the suitability of current testing methods exacerbated by limited availability to validated (in situ) biofilm monitoring tools. The Implementation Phase survey focused on regulatory clarity, with 58% of respondents rating current regulatory guidelines on antibiofilm agents as unclear or very unclear. Key challenges included limited international harmonisation, misalignment between academic and industrial priorities, and insufficient methods to support antibiofilm claims. Respondents called for clearer regulatory guidance, expert registers, training resources, and real-world data to support compliance and innovation. These findings will guide the Biofilm Alliance's development of recommended Regulatory Science tools and strategies to support effective sectors-wide biofilm management.

B077

Understanding the osmo-tolerance of Xerophilic Bacteria, Cronobacter sakazakii and the expressed Carbohydrate-active Enzymes.

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Abstract

The response of enzymes to low-water environments remains poorly understood. However, advancing this knowledge could have significant benefits for a range of biotechnological applications, where low water conditions are necessary. The characterization of desiccation-tolerant bacteria has revealed a diverse library of enzymes that can potentially be applied for such low water applications. In this study, we focus on the Gram-negative bacterium *Cronobacter sakazakii*.

To better understand the osmotolerance of *C. sakazakii*, the bacterium was exposed to hypersaline conditions, with significantly upregulated proteins identified through proteomic analysis. From these findings, a library of carbohydrate-active enzymes (CAZymes) were heterologously expressed to assess their capacity to withstand desiccation. Of particular interest was a family of glycoside hydrolase 13 enzymes. Members of this family exhibited tolerance to high salt concentrations and displayed significant activity at sodium chloride levels up to 1.5 M.

Current efforts are focused on developing methods to screen such enzymes in low water conditions, to define hydration thresholds for enzymes from Cronobacter and understand their potential for industrial re-application. An ELISA-based approach has been established using eGFP-linked carbohydrate-binding modules. Future work will focus on optimizing this method, as well as further characterizing the CAZymes derived from *C. sakazakii* using techniques such as molecular docking and crystallography.

B078

Bacterial community structure differs with the granule size in cryoconite on the Ny-Ålesund glacier

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Abstract

Cryoconite, the loose or granular dark-coloured sediment found on the surface of glaciers or ice sheets, consists of minerals, organic matter, and microbes. They result in different forms of aggregates, referred to as cryoconite granules, connected by extracellular polymeric substance produced by filamentous autotrophs, mainly cyanobacteria. There have been studies on bacterial communities across different granule sizes, but limited understanding of the Ny-Ålesund glacier granule. In this study, we used the bulk sample, i.e., surface cryoconite granules of Midtre Lovenbreen, to observe the bacterial community structure with different granule sizes. Different-sized pluristrainers (30 µm, 50 µm, 100 µm, 250 µm, 500 µm, 750 µm, and 1000 µm) applied in this research resulted in their respective size granules. Additionally, we measured the organic carbon content of each granule to explore the relationship between granule size and organic carbon content of each granule. Our results show there is a change of bacterial communities with the increase in size, as we find an increase of cyanobacteria and chloroflexi from lower to higher granules. In a similar way, organic carbon increases with granule size, which implies that the cyanobacteria and chloroflexi are the source of organic carbon in extreme cold environments. We find the observed values decrease with the higher granule size, which shows the number of different taxa (genera) decreases with higher granule size. This study demonstrates that the bacterial community structure differs with the increase of granule size, along with the increasing trend of organic carbon.

B079

LIPO-TOX TOXIN DETECTION TEST An Innovative and Rapid Liposome-Based Assay for Detection of Cytotoxic Membrane Damaging Toxin Producing Pathogenic Bacteria

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Abstract

Pathogenic bacteria are microorganisms that invade host tissues, causing diseases through their ability to produce toxins and enzymes, which harm living organisms. Toxins induce cytotoxicity and pathogenicity in host cells via various mechanisms, one of which is membrane damage. This study aims to develop a novel, rapid, easy, and cost-effective method to detect bacteria with such mechanisms. Hemolytic bacteria, including *Streptococcus pyogenes*, *Listeria monocytogenes*, and *Bacillus subtilis*, were used to assess membrane damage caused by their toxins and enzymes. Liposomes composed of soy lecithin and cholesterol were prepared and encapsulated with trypan blue. These liposomes were then exposed to bacterial toxins and enzymes, and the resulting hemolytic and lipolytic activity was evaluated both spectrophotometrically and visually. This test can detect bacteria that, although non-pathogenic under normal conditions and showing no pathogenicity at low doses, reach high concentrations in food (10^9 CFU) and become pathogenic in the body. It can also identify pathogenic bacteria proliferating above their infectious doses by detecting the membrane-damaging toxins' hemolytic-lipolytic activity. Unlike other tests that focus solely on bacterial growth or toxins, this test directly detects membrane damage at high bacterial doses, making it ideal for food safety. Additionally, bacterial hemolytic activity can be detected within two hours, making this method valuable for bacterial identification. In conclusion, this method allows the rapid, easy, and cost-effective detection of pathogenic bacteria with hemolytic and lipolytic activity in both raw and cooked foods.

B080

Integration of sulfur-oxidizing bacteria into biofilm-based filter for sustainable biogas purification

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Abstract

Biogas represents a promising renewable energy source, yet its utilization is hindered by the presence of hydrogen sulfide (H_2S), corrosive and toxic gas which in contact with water vapor strongly corrodes metal parts of the biogas process line. Developing efficient and sustainable desulfurization methods is therefore essential for reliable biogas purification and this study explores the usage of sulfur-oxidizing bacteria immobilized on a natural aluminosilicate carrier. Two chemolithotrophic bacterial strains, *Thiobacillus denitrificans* and *Paracoccus versutus* were successfully cultivated under controlled anaerobic and pseudo-anaerobic conditions to establish highly simplified and reproducible growth and maintenance protocols. Both strains separately were immobilized onto perlite particles and introduced into a laboratory-scale biofilter (glass column – *Ø* 10 mm, *h* 200 mm) for continuous biogas purification, and connected to 2 L laboratory scale anaerobic digesters. The system maintained moisture and nutrient balance by daily addition of nutrient solution. Preliminary experiments demonstrated stable bacterial activity and consistent desulfurization efficiency, achieving up to 88.9 ± 13.1% and 50.1 ± 21.6% H_2S removal over an operational period of 10 days for biofilter with *T. denitrificans,* and with *P. versutus*, respectively. After the experiment, composition of bacterial consortium on the bioparticles was analysed, revealing enrichment in bacterial species, and enabling identification of potential H_2S removing bacteria already present in the digesters. Further optimization will address long-term operation, prevention of biologging, and performance at larger scale. This work contributes to the development of environmentally efficient biofiltration system for industrial biogas treatment, emphasizing low-cost natural materials and accessible microbiological techniques.

B081

MICROBIAL PROFILE OF HOUSEFLIES FROM SELECTED AREAS IN AND AROUND KNUST

Ama Pinkrah

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Abstract

Background: Houseflies (*Musca domestica*) are well-known carriers of various microbial pathogens, posing significant public health risks. This study aimed to profile the microbial communities found on houseflies collected from three distinct environments within the KNUST area: the market, farm, and halls of residence.

Method: A total of 300 houseflies were collected for the study. Both external and internal microorganisms were isolated by rinsing the flies in saline and crushing whole specimens. Standard microbiological techniques such as biochemical tests, gram staining, microscopy and antimicrobial susceptibility tests were used to isolate, identify, and quantify bacteria, focusing on species such as *Staphylococcus, Klebsiella, Proteus* and *Micrococcus.*

Results*:* The highest bacterial load was observed in samples from the market (353.75 CFU/mL), followed by the farm (323.25 CFU/mL) and the halls (275.75 CFU/mL). Antibiotic susceptibility testing revealed that *Micrococcus* sp. isolated from the halls exhibited the greatest resistance, showing resistance to all 12 antibiotics tested followed by *Staphylococcu*s sp. In contrast, *Enterococcus* and *Micrococcus* species from the farm demonstrated the highest level of susceptibility to antibiotics. The highest bacterial load observed in the marketplace, indicating higher microbial contamination in this environment highlights the importance of monitoring microbial contamination and antibiotic resistance in environments frequented by houseflies.

Conclusion: The significant resistance observed in *Staphylococcus* sp. from the marketplace underscores the need for improved hygiene and infection control measures. Additionally, the findings contribute to a better understanding of the role of houseflies in the spread of antibiotic-resistant bacteria.

B082

Biotechnological Potential of Novel *Komagataeibacter* Strains from Mediterranean Vinegars Based on Genomic Analyses, and Chemical Characterization of their Produced Cellulose

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Abstract

Mediterranean ecosystems host diverse microbes with untapped biotechnological potential. Among microbial metabolites, bacterial cellulose (BC) stands out for its exceptional mechanical strength, water retention, and biocompatibility, surpassing plant cellulose and enabling applications in food, medical, and industrial sectors. *Komagataeibacter* is a key BC producer. This study aimed to isolate, characterize and assess the biotechnological potential of BC-producing strains from the “mothers of vinegar” during artisanal vinegar acetification.

Twenty-five acetic acid bacteria were isolated and identified via 16S rRNA gene sequencing and phylogenetic analysis. Genomes of five representative strains were sequenced to assess BC biosynthetic operons and secondary metabolite potential using antiSMASH. Phylogenomic analyses were performed. BC production was tested under defined conditions using glucose, fructose, and glycerol. The resulting BC films were characterized by FTIR and XRD.

Isolates belonged to *Komagataeibacter* (n=18) and *Acetobacter* (n=7), with several *Komagataeibacter* strains likely representing novel species (ANI <95%). Selected strains produced BC from all tested carbon sources, with yield and quality varying by strain and substrate. FTIR and XRD revealed structural and chemical differences among BC films. Genome mining confirmed cellulose operons in producers, while non-producers lacked essential genes or showed divergent operon structures.

Mediterranean vinegar ecosystems are valuable reservoirs of novel BC-producing bacteria, particularly *Komagataeibacter*. These findings highlight the importance of microbial biodiversity for biotechnological innovation and the potential of these strains to advance circular bioeconomy strategies in sustainable Mediterranean agro-food systems.

B083

Isolation, Characterization and Genomic Detection of Multidrug-Resistant Bacillus spp. From Spoiled Apples

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Abstract

The prevalence of Multidrug-resistant (MDR) bacteria in fresh produce is an emerging public health concern. Microbes found in spoiled apples may, however, harbour MDR bacteria. This study aimed to characterize and investigate the genomic detection of MDR *Bacillus* spp. isolated from spoiled apple samples collected from some selected states in Nigeria.

Bacteria were isolated from 150 spoiled apple samples collected across Lagos, Oyo, Osun, Ogun, and Kwara in Nigeria (30 per state). Isolates were identified using morphological and biochemical characteristics, and their antibacterial susceptibility was tested using the Kirby-Bauer disc diffusion method. The identity of the most prominent isolate was confirmed by molecular characterisation using the 16S rRNA sequencing. In addition, the Comprehensive Antibiotic Resistance Database (CARD) was used to detect antibiotic resistance genes (ARGs) present in the most prominent isolate.

Eighteen bacteria were isolated from the spoiled apple samples and identified as *Bacillus cereus* (11)*, Bacillus licheniformis* (6)*,* and *Bacillus subtilis* (1). The total heterotrophic bacterial count ranged from 1.49×10^4 CFU/g to 4.53×10^8 CFU/g, with Lagos samples exhibiting the highest microbial load. Antibiotic susceptibility profiles revealed diverse resistance patterns, with *Bacillus cereus* exhibiting the highest multiple antibiotic resistance (MAR) index of 0.5. Sequence reads from multidrug-resistant *Bacillus cereus* revealed 11 resistance genes:
bla1, bla2, tetB(P), vanT, vanW, vanY, mphL, fosB, fosB2, satA, and **qacJ.**

These results highlight the potential role of spoiled apples as reservoirs for antimicrobial resistance genes, representing a possible route for the dissemination of MDR bacteria and consequential public health concern.

Session : Exploring Microbiome Function

B084

rpoB: molecular marker to identify total and active bacteria in environmental samples?

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Abstract

Over the past three decades, the *16S rRNA* gene has served as the "gold-standard" for microbial community analysis. While it has yielded valuable insights, limitations such as multiple and variable gene copies per genome and poor correlation between rRNA and cellular activity have prompted the search for alternative markers. Housekeeping genes, particularly the RNA polymerase subunit B (*rpoB)* gene, offer promising advantages: *rpoB* is typically present as a single copy in bacterial genomes, improving phylogenetic resolution, and its transcriptional role allows for direct inference of cellular activity.

In this study, we present an in-silico evaluation of existing *rpoB* primers and propose a new primer set with enhanced coverage. The new primer set, was further applied and validated for both quantitative PCR (qPCR) and Illumina sequencing of co-extracted DNA and RNA (cDNA) from environmental samples, soil and sediments.

They demonstrate high qPCR efficiency (>80%), detect ~9-fold and ~5-fold fewer gene copies per gram of soil and sediment, respectively, than *16S rRNA*, consistent with expectations based multiple gene copy per genomes. Furthermore, the primers are compatible with Illumina sequencing of both total and transcriptionally active bacterial communities in complex environmental samples. When using our newly curated reference database of over 300,000 unique, verified *rpoB*sequences, taxonomic assignment yielded ~15–28% unassigned reads, comparable to results obtained using *16S rRNA*, demonstrating that *rpoB* could be a valuable complementary marker to identify total and active bacteria in environmental samples.

B085

Optimising the Adaptation Phase and Investigating the Stability of Cattle Rumen Fluid in a Dual-Flow Fermenter System using a 50:50 Forage-to-Concentrate Diet

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Abstract

Understanding when rumen fluid stabilises in dual-flow fermenter (DFF) systems is essential in improving the accuracy of *in vitro* rumen studies. This study investigated the adaptation period and microbial stability over a 30-day DFF run inoculated with rumen fluid from steers fed a 50:50 forage-to-concentrate diet. Fermenters were maintained at 39 degrees under anaerobic conditions with continuous buffering (pH 6.5 - 7.5) and were monitored for methane (CH_4), oxidation-reduction potential (ORP), volatile-fatty acids (VFAs), dry matter (DM) and organic matter (OM) degradation, alongside bacterial, archaeal and fungal community composition (16S/28S rRNA sequencing).

Methane production displayed four phases: early stabilisation (days 1-10), peak activity (day 15), transient decline (days 16-21) and steady state (days 21-30). Acetate dominated the VFA profile, while butyrate and propionate peaked during active fermentation. ORP fluctuated between -300mV and -100mV before stabilising near -200mV after day 15, indicating a balanced syntrophic community. OM and DM degradation stabilised after two weeks, supporting steady fermentation dynamics. Microbial diversity decreased post-inoculation, then recovered as communities converged toward a consistent structure by days 21-30. PERMANOVA confirmed day as the main driver of community variation ($p < 0.001$).

Overall, DFF systems required approximately 15 days for microbial and functional parameters to stabilise. Although the adapted community differed from the initial inoculum, it maintained core metabolic functions. treatments should therefore be applied only after this adaptation phase to avoid confounding early microbial shifts with treatment effects.

B086

Optimising swab collection methods for the assessment of host-microbiome interactions

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Abstract

Background

The skin microbiome is implicated in various skin conditions, yet its role in sensitive skin syndrome (SSS) is uncharacterised. Due to its low biomass environment it is important that optimised sampling approaches are established, to fully understand the interplay between the microbiome and health.

Methods

To test recovery, cotton, rayon and flocked swabs were immersed in 10_8 CFU/mL of *Staphylococcus epidermidis* and transferred into BHI media. Swab aliquots were plated, and colonies were counted after 24 hours.

To evaluate transport media, flocked swabs were immersed in 10_8 CFU/mL suspensions then transferred into BHI, PBS and in-house and commercial liquid amies solution. Colony counts and DNA extraction yields were assessed after immediate collection, 3 hours at room temperature, and 24 hours at 4°C and -20°C. This was repeated for C*utibacterium acnes* and a skin bacteria model.

Results

Flocked swabs are more efficient than rayon and cotton swabs, at capturing S*. epidermidis* and *C. acn*es. All swabs were better at capturing *C. acnes* than *S. epidermidis*, with rayon swabs exhibiting the highest percentage increase of 460.4%.

Between the four media types, BHI shows a consistently higher DNA recovery rate among all conditions, while media efficiency in bacterial capture is comparable. PBS and in-house liquid amies are significantly limited in DNA recovery compared to viable bacterial retention.

Conclusion

Our findings highlight the importance of suitable collection methods for the assessment of low biomass samples. An accurate representation of microbial communities will allow for comprehensive interpretations of host-microbiome functions.

B088

Blurring osmoregulatory boundaries: Microbial diversity and survival strategies across a hypersaline gradient

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Abstract

Hypersaline environments are extreme ecosystems where high salt concentrations, nutrient scarcity, and other factors limit microbial diversity and shape the structure of prokaryotic communities. To cope with these conditions, microorganisms employ osmoregulation mechanisms. Traditional studies on osmoadaptation have focused on culturable microorganisms, but little is known about these strategies at community level. To address these questions, marine salterns are ideal environments, as they consist of ponds with salinities ranging from that of seawater to saturation.

In this study, the Isla Cristina and Isla Bacuta (Huelva, Spain) salterns were analyzed along a salinity gradient from 13% to 44% (w/v) to: (1) study microbial diversity as a function of salinity; (2) detect inflection points in community dynamics along the salinity gradient; (3) examine osmoregulation mechanisms and their distribution between *Archaea* and *Bacteria*.

A total of 34 Illumina shotgun metagenomes were obtained, from which 1,478 MAGs were recovered. These were compared with 546 MAGs from nearby marine areas and more than 800 reference genomes of cultured halophiles. The results showed that diversity did not decrease drastically with increasing salinity, as expected; instead, it remained relatively stable at intermediate salinities, with 25% (w/v) appearing to be the inflection point where community dynamics changed. Moreover, a dominance of archaea was observed, along with an increase in “salt-out” strategy, which is commonly associated with the *Bacteria* domain. These findings provide a new perspective on diversity in hypersaline environments and highlight the role of archaea as osmolyte producers, blurring the phylogenetic boundaries in osmoregulation strategies.

B089

Models to determine functions of the oral microbiome for personal care products safety assessments

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Abstract

With the increase of personal care products targeting the microbiome for cosmetic benefits, there is a need to ensure that perturbations of the microbiome do not have potential unwanted consequences on the health of consumers. Safety assessments of new technologies currently rely on monitoring changes of microbial taxa in clinical studies, but it would be desirable to characterise the protective functions of the microbiome.

The role of commensal bacteria against pathogens can be effectively studied using a combination of *in vitro* and *in silico* models in the context of dental caries. A continuous bioreactor-based *in vitro* model utilizing hydroxyapatite coupons as tooth enamel analogues was inoculated with four early colonizer commensals alongside the caries-associated *Streptococcus mutans*. Together with individual-based modelling of biofilm growth, this showed that *S. mutans* dominated at low pH. However, as bacterial metabolism was represented using stoichiometric equations for growth derived from literature, ongoing work aims to reconstruct genome-scale metabolic models for each species to enable more accurate community modelling.

Additionally, the resilience of a healthy microbiome - a key indicator of oral health- can be investigated in vivo through experimental gingivitis models. A study was designed collecting samples including during the recovery phase for metagenomics and meta-transcriptomics samples alongside clinical indices. Shotgun sequencing facilitates the identification of functional genes and pathways, while meta-transcriptomics reveals their activity.

Taken together, these examples demonstrate the value of integrating *in vitro, in silico*, and *in vivo* models to elucidate microbiome functions relevant to safety assessments.

B090

Investigating transcriptional and phenotypic interactions between Trichomonas vaginalis and the vaginal microbiome.

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Abstract

The vaginal microbiome, primarily dominated by lactobacilli, is important for women's health. Sequencing has identified that the vaginal microbiome can be broadly categorized into at least 5 community state types (CST). CST I, II, III, and V are dominated by *Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus iners,* and *Lactobacillus jensenii,* respectively. These bacteria are important for the prevention of pathogen colonization, and a shift to the CST IV (depleted in lactobacilli) has been associated with an increased risk of preterm birth, cervical cancer, and an increased risk of sexually transmitted infection (STI) acquisition. *Trichomonas vaginalis* is the most prevalent treatable STI worldwide, and the World Health Organization approximated 156 million new cases of trichomoniasis in 2020. The relationship of *Trichomonas vaginalis* with other members of the vaginal microbiome is poorly understood. **F**ew studies have comprehensively incorporated phenotypic and transcriptomic data in the context of host-pathogen-commensal dynamics in the vaginal microbiome. Using a vaginal epithelial cell co-culture model, we investigate the response of different CST/*T. vaginalis* combinations using both transcriptomic and functional analysis. Specifically, we measure cell/colony counts over time, epithelial barrier integrity, cytotoxicity, and lactic acid production. Additionally, we are analysing the expression of key genes from the host, *T. vaginalis,* and each lactobacilli strain. Taken together, this approach will reveal important mechanisms underpinning interactions within the vaginal microbiome.

B091

Culturing the Wild Microbiome: Designing Host-Derived Probiotics to Understand Behaviour in Wild Birds

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Abstract

The gut microbiome plays a crucial role in shaping host physiology and behaviour via the microbiome-gut-brain axis (MGBA), yet despite the critical importance of animal behaviour and cognition in responding to environmental change, it remains poorly understood in wild animals. One approach to address this challenge is to manipulate the gut microbiome in an ecologically valid way. By isolating host-derived symbiotic microbes involved in the MGBA, they can be administered to test if their increased abundance in the host influences physiology and behaviour. This project investigates how natural variation in gut microbiota influences behaviour in widespread passerines, the great tit (*Parus major*) and blue tit (*Cyanistes caeruleus*). The first question asks, which host-derived gut microbes can be isolated and functionally characterised across habitats, age groups, and species? To address this, culturomics will be employed to isolate host-native bacteria, using different selective media, aerobic/anaerobic conditions, temperature and pH ranges. Whole genome sequencing will characterise the isolates, with many expected to be *Lactobacillus* and *Bifidobacterium* genera, and identify genes linked to host interaction and neuromodulatory genes expected to influence MGBA function. Supplementation with selected host-native strains will be tested in captive great and blue tit nestlings. It is predicted that microbial enrichment will modulate cognitive (problem-solving, learning) or behavioural traits (neophobia) via MGBA-mediated mechanisms. By elucidating how microbiota modulates behaviour, our work offers new perspectives on the MGBA, its ecological significance, and may provide the basis for future microbiome-based interventions supporting adaptive responses in wild avian species.

B092

Effects of Commercial Phytogenic Feed Additives on Rumen Function, Microbial Communities and Methane Production: An *In Vitro* Study

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Abstract

Phytogenic feed additives are gaining prominence as sustainable strategies to support the health and productivity of ruminants. This study investigates six phytogenic feed additive products (AHV 1-6), two different combinations of products (AHV 7 and 8) and three core ingredients (AHV 9-11) produced by AHV International, a global leader in phytogenic solutions for ruminant health, using a ruminal *in vitro* batch fermentation methodology. Rumen fluid diluted with saliva mimic buffer, diet substrate and test compounds were incubated anaerobically and allowed to ferment at 39°C. pH was monitored, and samples were taken at defined time points (0, 4, and 24 hours) for the quantification of methane, ammonia, and volatile fatty acids (VFAs), as well as for DNA extractions to facilitate downstream analysis. AHV 1-11 did not significantly impact pH during fermentation. Methane responses were compound-specific, with some products (AHV 2, 3, and 5) increasing methane production by 8-33% compared to the control ($p < 0.05$). Conversely, two core ingredients (AHV 9 and 11) tended to reduce methane output. Preliminary ammonia quantifications demonstrated considerable variability across biological replicates. These initial findings suggest potential optimisation of phytogenic inclusions to mitigate any methane-enhancing effects. Ongoing work includes full ammonia and VFA analyses, alongside 16S sequencing, to investigate microbial shifts underpinning fermentation changes. Dose-response studies will be crucial in validating trends and informing optimal inclusion strategies. This research aligns with One Health priorities by reducing dependence on antimicrobials by supporting ruminant health and production.

B093

Ulvan-degrading enzymes from North Ronaldsay sheep for marine biomass valorisation and sustainable agriculture

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Abstract

Green macroalgae (*Ulva* spp.) are an abundant renewable resource, rich in the sulphated polysaccharide ulvan, whose degradation offers opportunities for marine biomass valorisation and feed additive development supporting sustainable agriculture. However, ruminants lack the gut microbiota capable of fermenting ulvan, and hydrolytic tools for its saccharification remain underdeveloped.

The North Ronaldsay sheep has evolved to subsist almost entirely on *Ulva* spp., and its gut microbiome may therefore present a natural and unique reservoir of ulvan-active enzymes. This study aims to identify, characterise and produce ulvan-degrading enzymes from the rumen microbiome of the North Ronaldsay sheep for use in enzymatic feed supplements that enhance ruminal fibre digestion, redirect fermentation pathways, and reduce hydrogen availability for methanogenesis, thereby lowering enteric methane emissions.

Faecal samples from four sites on North Ronaldsay island (Orkney, Scotland) were cultured anaerobically in RMO2/GenRFV media. Partial 16S rRNA gene sequencing was performed on 250 isolated colonies. An NCBI BLAST search identified a range of bacterial genera, with obligate anaerobe *Bacteroides* being of interest due to the anaerobic nature of the rumen environment. Enzymatic screening for ulvan lysase, sulfatase, cellulase and glycosidase activity will be performed, and genetically diverse isolates with multi-enzyme activity subjected to whole genome sequencing and annotation. CAZyme genes predicted to be involved in ulvan hydrolysis will be cloned, expressed and purified, with enzyme activity validated and optimised. The RUSITEC system will then evaluate enhanced digestibility of *Ulva* spp. and reduced methane emissions from ruminants supplemented with novel enzymes during *in vitro* livestock feed fermentation trials.

B095

Dissemination and variation of selected Antibiotic Resistant Genes (ARGs) in differently fertilised soils and the role of heavy metals as Anti Microbial Resistance (AMR) co-selectors of greenhouse grown lettuce.

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Abstract

ARGs are prevalent in agricultural soils due to organic fertiliser amendments. To tackle AMR it is important to understand how the presence of heavy metals influence AMR entering the food chain and the impact of heavy metals as a selective pressure for AMR dissemination.

The objective of this greenhouse trial was to evaluate the effects of two organic fertilisers, a chemical fertiliser and topsoil on soil AMR and identify how heavy metals may exert selective pressure on AMR. High-throughput qPCR was used to quantify the ARGs and elemental compositions determined using SEM-EDX.

AMR levels in chemically fertilised soils displayed a pattern where the first week had highest levels in comparison to the following weeks. The final six weeks, along with the death of lettuce; displayed reduction and stabilisation of AMR levels.Digestate fertilised soil and topsoil, displayed similar patterns of AMR level changes with highest levels observed in week 1 and levels stabilising towards the harvest.

It is the biofertilised soil that displayed a difference in the patterns of AMR levels over time with highest AMR levels in week 8 and lowest levels in week 6. It is also the only fertiliser to display a negative correlation between the ARGs.

Digestate and topsoil saw significant AMR reductions over the course of the trials. Interestingly, the two heavy metals observed Pd and Fe were positively correlated to the ARGs analysed. Early analysis shows that digestate shows promise as an organic fertiliser and a positive correlation between heavy metals and ARGs.

B096

The Impact of the Oral Ecosystem: A Bioinformatics Framework for Metagenomic Insights of Oral and Tonsillar Environments

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Abstract

Introduction: Fluctuations in the diverse oral microbiome (OM) is increasingly associated with oral health and disease, while also having systemic implications. Our knowledge of the OM's composition is based on the hundreds of amplicon-sequencing-based studies from the last decade. Alternatively, shotgun metagenomics (SM) can give a more representative OM interpretation, identify novel organisms, and explore genetic factors beyond the microbiome. However, the data remains challenging to analyse.

Aim: To utilise existing databases and generate a new tonsillitis clinical dataset to develop a reproducible bioinformatic toolkit for investigating the roles of OM members and their metagenomic profiles in health and disease.

Methodology: A previous meta-analysis of oral-disease-related studies serves as a foundation for a subsequent bioinformatic pipeline built to study the oral and tonsil SM data. Sequences will undergo taxonomic profiling, gene annotation, functional prediction, and comparative analyses. Unique tonsil isolates (UIs) were identified using 16S-ITS-23S rRNA sequencing. Machine learning algorithms will be trained with metagenomic and questionnaire data to predict patterns across all datasets.

Results & Conclusions: The tonsillitis study recruited 50 patients and 31 healthy controls. Thus far, *Streptococcus pyogenes* has been isolated exclusively from patients, and *Neisseria* species were most prevalent amongst healthy UIs. SM analyses could improve our understanding of how oral taxonomical and genetic factors influence the upper respiratory tract's microbiome, clarifying the dynamic interactions between these microbial ecosystems and their roles in health and disease. Overall, this study will produce intuitive pipelines, and the resulting toolkit could increase accessibility to complex datasets investigation.

B097

Characterisation of strain level mechanisms driving host interaction in the vaginal microbiome

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Abstract

The composition of the vaginal microbiome plays a crucial role in maintaining vaginal health and influencing obstetric outcomes. *Lactobacillus*-dominated communities are typically associated with health, contributing to pH regulation, prevention of pathogen adhesion, and modulation of immunogenic responses. To date, the communities of microbes in the vagina have been defined at a species level and grouped into community state types (CSTs). These CSTs are more often dominated by just a single species and sequencing studies have associated changes in these with different health outcomes. There is now a growing appreciation that the relationship between the vaginal microbiome and health needs to take a strain resolved approach. Within any given species, the diversity of functional potential can be high. For *Lactobacillus jensenii*, which dominate CST-5, it was shown that strains from either full-term or preterm pregnancies can be phylogenetically distinct. These strains differed in genes related to cell surface structure and metabolic capacity, indicating that not all *Lactobacillus* strains play equivalent roles in the vaginal microenvironment.

In this study, we investigate the functional differences driving host interaction within *L. jensenii* strains isolated from either full-term or preterm pregnancies. Using co-culture assays with human vaginal epithelial cells (VK2), we assess differences in bacterial growth, epithelial adherence, cytotoxicity, and cytokine release. Strains isolated from preterm pregnancies had increased growth rates compared to their full-term equivalents and enhanced colonisation of vaginal VK2 epithelial cells. This study reveals host-microbe interactions within the vaginal microbiome are driven by strain level differences.

B098

Oh poop! Pawsitive selection in the resistome and microbiome of the canine gut in the first year of life

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Abstract

Antimicrobial resistance (AMR) poses an escalating challenge to human and veterinarian spaces, yet little is known about its early development within companion animal microbiomes. This study explores the evolution of AMR genes (ARGs) in the gut microbiome of Labrador Retriever puppies across their first year of life. Using shotgun metagenomic sequencing, we profiled microbial community composition and ARG prevalence at 4, 7, and 12 months of age. Preliminary data revealed that puppies shared highly similar microbial profiles at 4 months, dominated by *Segatella copri* (63 ± 26%) and *Megamonas uniformis* (6 ± 3%). Despite this compositional homogeneity, the resistome exhibited wide diversity, averaging 12 ARGs per million bases sequenced, primarily β-lactamase, tetracycline, and macrolide resistance genes. Rarefaction analysis demonstrated that approximately 1 Gbp of sequence depth is necessary for full resistome characterisation, underscoring the hidden diversity of AMR within animal microbiomes. Notably, ARG abundance appeared uncoupled from major taxonomic shifts, suggesting environmental exposure may be key drivers. The low abundance of putative pathogens detected amongst the samples (e.g., *Campylobacter*, *Helicobacter*) indicates these genes are harboured largely by commensal taxa. These findings provide baseline data for understanding how AMR evolves during host development and highlight the potential for early-life establishment of resistance reservoirs in domestic dogs. Improved surveillance of animal microbiomes could enhance predictive models of resistance transmission between companion animals and humans.

B099

Age-Related Skin Microbiome Dynamics: From Community Profiling to Strain-Level Analysis

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Abstract

Recent research demonstrates important shifts in the skin microbiome with age, which may be linked to skin ageing processes. Nevertheless, very few studies have provided strain-level characterisation of the ageing skin microbiome, and functional assessment of host-microbe interactions remains limited. This work applies Oxford Nanopore sequencing for metagenomic profiling and for whole genome characterisation of bacterial isolates to evaluate their role in ageing. High-resolution profiling of young (<35 y, n=20) and old (>60 y, n=22) adults revealed that body site (forehead, arm, and foot) was the primary driver of bacterial community structure, with age as a secondary factor. Age-related changes included increased alpha diversity, distinct beta diversity, reduced *Cutibacterium* and *Staphylococcus* abundance, and enrichment of opportunistic taxa. Given the observed reduction in *Staphylococcus* abundance with age, we next assessed intraspecies diversity of *S. epidermidis*, a keystone skin commensal, to investigate potential age-associated phenotypes. Pangenome analysis revealed phylogenetic diversity among *S. epidermidis* strains within individuals. Interestingly, age did not significantly influence cluster distribution. Gene presence/absence patterns related to antimicrobial resistance, extracellular matrix degradation, and biofilm production were heterogeneous among strains and showed no correlation with volunteer age. These findings underscore the importance of exploring microbial diversity beyond species level and the need to identify factors driving such diversity, including the interplay between host genetics and the skin microbiome. Together, our work provides a foundation for understanding how bacterial strains contribute to skin ecosystem dynamics across the lifespan, paving the way for developing microbiome-based interventions to support skin ageing.

Session : Infection Forum

B100

‘Prophylaxis-lite’, Diagnostic-Driven Infection Management in Febrile Neutropenia

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Abstract

Febrile neutropenia (FN) is a life-threatening complication in patients receiving chemotherapy or haematopoietic stem cell transplantation, often requiring broad-spectrum antibiotics. To mitigate antimicrobial resistance (AMR), St Bartholomew’s Hospital discontinued ciprofloxacin prophylaxis in 2019, adopting a diagnostic-driven approach supported by antimicrobial stewardship (AMS). This retrospective audit reviewed 86 haemato-oncology patients (acute leukaemias, myelodysplastic syndrome, or stem cell transplantation) admitted with FN between 2023-24. Empirical therapy followed local guidelines: piperacillin-tazobactam ± amikacin, or meropenem for allergy, with vancomycin reserved for resistant Gram-positive infections. Microbiology records were analysed to determine culture results, susceptibility patterns, and antibiotic decision-making.

Of 83 patients receiving empirical antibiotics, 42 positive cultures were identified (36 blood, 5 urine, 1 sputum). Eleven were contaminants, leaving 31 clinically relevant isolates. Gram-negative organisms predominated, notably *E. coli* and *Klebsiella* spp. Susceptibility data influenced prescribing in 26/31 (84%) cases: 9 escalations (resistance or clinical deterioration), 9 de-escalations (usually to co-amoxiclav or ciprofloxacin), and 6 where empirical therapy was already appropriate. Four cases were unaffected by culture data. Confirmed AMR was rare, with one Vancomycin-Resistant Enterococcus and one possible resistant isolate; several AmpC and ESBL producers were detected, but no carbapenem-resistant organisms.

A diagnostic-led, stewardship-based approach to FN safely reduced unnecessary broad-spectrum use, maintained low resistance rates, and supported timely de-escalation. This 'prophylaxis-lite' model demonstrates that microbiology-guided prescribing can protect patients and preserve antibiotics, offering a sustainable framework for infection management in high-risk haematology settings.

B101

Public health surveillance of *Vibrio cholerae* in travellers' returning to the United Kingdom, 2004-2024.

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Abstract

Introduction. *Vibrio cholerae* is a diverse species of bacteria causing watery diarrhoea, vomiting, and stomach cramps, and is the aetiological agent of cholera. Despite the global upsurge in cholera notifications, systematic analysis of national and international surveillance data describing the microbiology and epidemiology of *V. cholerae* is sparse.

Methods. We reviewed the microbiology and epidemiology of *V. cholerae* isolated from travellers returning to the UK. Epidemiological and microbiological data generated via traditional biochemistry, serology, PCR and whole genome sequencing, from all human isolates of *V. cholerae* detected from 2004-2024 were retrospectively analysed.

Results. There were 984 notifications of *V. cholerae* from 2004-2024, of which 27.0% belonged to serogroup O1. There were over 180 different sequence types (STs), where ST69 was predominant (28.2%). The highest number of isolates was in 2010 (*n*=74), while the lowest was in 2020 (*n*=8) and 2021 (*n*=4). Children under the age of 10, the middle aged, and the elderly population were most susceptible to infection and 51.6% of the cases were male. There was a seasonal peak in August. Travel was reported by 92.9% of cases, with India, Pakistan and Kenya the most frequently reported.

Conclusions. Public health surveillance is essential to assess the impact of investment in WASH initiatives for the prevention of cholera. To assess the risk to food safety and determine the clinical burden, we recommend widespread molecular testing to monitor the emergence of *V. cholerae* in UK waters due to climate change, including testing of faecal specimens from non-travellers with gastrointestinal symptoms.

B102

Surveillance of Genomic Diversity and Antimicrobial Resistance in Enterotoxigenic *Escherichia coli* in England, 2015-2023.

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Abstract

Introduction: Enterotoxigenic *Escherichia coli* (ETEC) is one of the leading causes of gastrointestinal infections globally, primarily affecting children in low-/middle-income countries and travellers to endemic regions. Despite the clinical and public health burden, ETEC surveillance in England remains limited. This study aimed to utilise available data to describe the epidemiology, genomic diversity and antimicrobial resistance (AMR) of ETEC in England.

Methods: From 2015 to 2023, there were 587 ETEC isolates cultured from faecal specimens referred to UK Health Security Agency and sequenced to determine sequence type, serotype, virulence and AMR profile, integrated with epidemiological data from referral forms.

Results: The number of ETEC notifications increased annually, with a 35.5-fold increase from 2015 to 2023. 51.7% of cases were females and 48.3% males, with the highest proportion belonging to the 50-59 age group (18.6%). 49.5% of cases were travel-associated, with Egypt, Pakistan, India, and Turkey being the top travel destinations. 139 STs and 132 serotypes were identified, with the most common being ST4 O6:H16 (n=74) and ST182 O169:H41 (n=66). Genome-derived AMR data revealed widespread resistance to fluoroquinolones and β-lactams, including 3rd generation cephalosporins, over 40% of isolates being resistant to three or more antimicrobial classes.

Conclusions: We observed rising notifications of multidrug-resistant ETEC over the last decade, mainly associated with travellers' diarrhoea. Nationwide expansion of ETEC PCR-based diagnostics and stronger collaboration with public health agencies and genomic data sharing at a national and international level, are critical for improving surveillance and assessing the true burden of ETEC locally and globally.

B103

Microbiology and epidemiology of enteroaggregative *Escherichia coli* isolated from UK residents in England, 2016-2023: what are the risks to public health?

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Abstract

Introduction. Following two large foodborne outbreaks of enteroaggregative *Escherichia coli* (EAEC), UKHSA implemented enhanced surveillance strategies for EAEC. We reviewed the microbiological typing and demographic data of cases diagnosed from 2016-2023. The aim was to determine the community burden of gastrointestinal disease caused by EAEC and assess the risk to public health.

Methods. PCR-positive diarrhoeagenic *E. coli* faecal samples were referred to UKHSA for confirmation and culture. EAEC isolates were sequenced on the Illumina NextSeq platform. Sequence type (ST), serotype and antimicrobial resistance (AMR) profile was derived from the genome sequence. Age, sex and travel histories (where available) were linked to the typing data.

Results. There were 1402 notifications of EAEC, exhibiting a five-fold increase in diagnoses from 93 in 2016 to 524 in 2023. The most common STs were ST34 (14.4%), ST10 (13.2%), ST200 (13.1%), ST678 (7.2%), and the most common serotypes were O92:H33 (9.3%); O175:H31 (5.6%); and O99:H10 (5.6%). Most cases were female (54.5%) and/or were aged <10 (28.2%), within which 77.3% were <5 years old. 79% of cases reported foreign travel within 7 days of onset of symptoms. AMR was detected in 74.5% isolates with resistance to fluoroquinolones (57.8%) and beta-lactams (57.6%) antibiotics being the most common.

Conclusions. Given the burden of disease caused by EAEC in the community, the high proportion of infections in children and travellers, the risk of the emergence of hybrid pathotypes, and the high proportion of AMR, we concluded that EAEC should be part of the diagnostic algorithm in the UK.

B104

A classical analysis “on steroids”: unveiling genome-wide selection pressures on a bacterial pathogen resolved at single nucleotide resolution

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Abstract

Habitats select for adapted microorganisms. *Pseudomonas aeruginosa* is a versatile bacterium that can rapidly microevolve and settle into different ecological niches. It is also a major burden to public health. A fundamental question is therefore: which genes are important for the infectious lifestyle? Previous efforts to address this have combined experimental and analytical approaches, the latter of which are dominated by running simulations on very large datasets. However, these simulations have strict data requirements and are not well-suited for multi-niche bacteria such as this one. To address this issue, we developed a simulation-free approach using an augmented d_N/dS algorithm. Our data resolve selection pressures to the single amino acid level. The data revealed a novel association between quorum-sensing deficiency (loss of lasR function), diminishing biofilm formation ability (gene lost in the *psl* cluster) and the acquisition of mucoidy, which has clinical significance to the people with cystic fibrosis (CF). By contrasting the genes associated with habitat-specific selection pressure in CF-associated (“clinical”) isolates and “environmental” isolates, our results revealed that habitat-specific selection pressure concentrates on metabolic branchpoints. This agnostic d_N/d_S approach can provide evolutionary insights that are free from many lifestyle assumptions and can be leveraged for high-powered investigations on any species with a scarcity of available genomes and/or variable evolutionary rates.

B105

ANTI-KINETOPLASTID PROPERTIES OF SIPHONOCHILONE, A FURANOSESQUITERPENOID EXTRACTED FROM SIPHONOCHILUS AETHIOPICUS

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Abstract

The order Kinetoplastidae, especially members of the genera *Leishmania* and *Trypanosoma,* contain parasites of significant medical and veterinary importance for which new drugs are urgently needed. *Siphonochilus aethiopicus*, also known as African ginger, is regarded as one of the major therapeutic plants in the world. We investigated the activity of siphonochilone, one of the main active compounds isolated from this plant, against *Trypanosoma brucei brucei*, *T. evansi, T. equiperdum, T. congolense, Leishmania major* and *L. mexicana* using resazurin-based assays. Activity against all these parasites was in the low micromolar range, with the lowest EC_{50}, for *L. major*, just submicromolar at 0.92 ± 0.05 µM; the compound did not affect the intraerythrocytic cycle of *Plasmodium falciparum*. Cytotoxicity towards human Foreskin Fibroblast (HFF) cells was low at 174 µM, resulting in high Selectivity Index values. Siphoniochilone dose-dependently cleared trypanosome and *Leishmania* cultures between 24 and 96 h. We thus conclude that siphonochilone shows potent and specific activity against kinetoplastid parasites but not *P. falciparum* or human cells.

B106

A new approach to measuring the antimicrobial efficacy of hypochlorous acid.

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Abstract

Background:

Hypochlorous acid (HOCl) is a powerful biocide. It exists in equilibrium with hypochlorite, with HOCl dominant below pH 7 and OCl^- dominant above pH 7. Although the efficacy of HOCl has been demonstrated using MIC and MBC assays, reported values are often inconsistent, likely due to rapid quenching. We developed a defined, pH-controlled protocol to measure MIC/MBC of pure HOCl against clinical strains of *Pseudomonas aeruginosa*.

Methods:

P. aeruginosa strains were grown in Nutrient Broth, standardised and exposed to HOCl. Standard MIC assays used the broth microdilution method. Bactericidal activity was confirmed by a time-kill assay. We used a modified saline (at pH 4) as a diluent and added glucose post-treatment as a nutrient source. The MIC was determined by measuring metabolic activity via resazurin fluorescence, while the MBC was confirmed through plating.

Results:

In standard MIC assays HOCl was quenched by the growth media, showing no activity despite confirmed rapid killing from time kill assays. Results from the modified protocols, showed measurable MICs and bactericidal MBCs, with reduced efficacy at pH >7.5. When tested under controlled conditions at a favorable pH (pH 4) and without complex growth media, HOCl produced clear MIC and MBC values and showed consistent bactericidal activity, which declined at higher pH.

Conclusion

These findings demonstrate that MIC methods/protocols designed for antibiotics are unsuitable for reactive biocides like HOCl. Appropriate assays are essential for accurate potency assessment and clear mechanistic interpretations.

B108

Accelerating diagnostics: Development of a loop-mediated isothermal amplification (LAMP) assay for rapid identification of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a major pathogen in cystic fibrosis (CF) lung infection, but can also cause infection in the urinary tract, wounds and other sites. There is emphasis on rapid identification to enable early initiation of appropriate antibiotic therapy, which is particularly important in people with cystic fibrosis (pwCF), where chronic *P. aeruginosa* infection is associated with increased morbidity and mortality. Loop-mediated isothermal amplification (LAMP) was developed as a cheaper, more sensitive and faster alternative to other molecular assays. This study aimed to develop a LAMP assay targeting the *oprL* gene in *P. aeruginosa*, and to determine the potential use of saliva to detect *P. aeruginosa* in pwCF. Reactions were monitored through real-time fluorescence. Both purified DNA and samples prepared using a simplified thermal lysis step were tested. The assay successfully demonstrated successful detection across multiple sample types, including saliva, with both direct sample loading and a quick (up to 15 minutes) thermal lysis step. The limit of detection using extracted DNA was 0.87 fg, and for thermally lysed spiked saliva it was 1.4×10^3 cfu/mL, at an average detection time of 17 minutes. Evaluating the assay on 38 *P. aeruginosa* isolates showed 100% positive detection, whilst testing of an additional 58 non-*Pseudomonas* species showed no amplification. Further work will test clinical samples to validate the assay and assess the potential use of saliva as an alternative to sputum in pwCF.

B109

ChiA, a major virulence factor of Crohn's disease associated-adherent and invasive *Escherichia coli* (AIEC) that could be counteracted with chitinous elements

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Abstract

Crohn's disease (CD) is a chronic inflammatory bowel disease characterised by a disturbance in intestinal microbial communities. The intestinal dysbiosis observed in CD patients is often marked by an increase in adherent and invasive *Escherichia coli* (AIEC), present in 21 to 63% of patients, *vs* 0 to 19% in healthy individuals. AIEC adhere to and invade intestinal epithelial cells (IECs) and replicate/survive in intestinal macrophages. Our study brings a description more complete of the virulence factor ChiA (endochitinase-A), its polymorphisms and its action. Metagenomics analysis reveals that *chiA* gene is significatively and specifically more abundant in the microbiota of CD patients *vs* healthy subjects (100-fold higher, $p<0.01$). Further studies performed in mice model combined with *in vitro* experiments revealed that ChiA plays a role in 1) the bacterial colonization in intestinal mucosa, 2) the mucus degradation ($p<0.05$), enabling AIEC bacteria to gain better access to IECs ($p<0.05$), 3) the adhesion of AIEC bacteria to IECs *via* the eukaryotic transmembrane protein CHI3L1 ($p<0.001$), 4) the crossing the epithelial barrier ($p<0.01$), 5) the invasion into mesenteric lymph nodes ($p<0.01$) and payer's patches ($p<0.05$), and 6) the macrophage entry of AIEC bacteria ($p<0.001$). Our findings identify ChiA, and particularly its pathogenic polymorphism, as a major virulence factor of AIEC. In a colitis-mice model, chitin and chitosan administration reduced significatively the disease activity score ($p<0.05$) and the AIEC colonization in ileum and colon compared to the untreated group ($p<0.05$). Targeting ChiA with chitin-derived compounds may represent a promising strategy for controlling AIEC colonization.

B110

Broad-Spectrum Habits Die Hard: Persistent Gaps in Antimicrobial Stewardship and Pneumococcal Vaccination Over a Decade

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Abstract

Background

Invasive pneumococcal disease (IPD) remains a major cause of morbidity in adults, despite the availability of effective vaccines and evidence-based antimicrobial guidelines. Understanding local patterns in management and prevention can inform stewardship and immunisation strategies.

Objectives

To evaluate clinical management, antimicrobial use and pneumococcal vaccination practices among adults with *Streptococcus pneumoniae* bloodstream infections (BSIs) over a ten-year period.

Methods

A retrospective review of laboratory-confirmed pneumococcal BSIs at a tertiary hospital (2014–2023) was conducted. Clinical and microbiological data were extracted from electronic records to assess antimicrobial susceptibility, de-escalation practices, complications, ICU admissions, and documentation of pneumococcal vaccination recommendations.

Results

Among 176 cases identified, the median age was 69.5 years, and 56% were aged ≥65 years. Penicillin susceptibility remained high (85%), yet only 25% of eligible patients were de-escalated from empiric broad-spectrum therapy. Complications occurred in 15% of cases and ICU admission in 10%. Of all patients, 22% had pneumococcal vaccination documented at discharge. Serotype 8 predominated, with non-vaccine serotypes comprising 23% of isolates.

Conclusions

Despite favourable susceptibility patterns, narrow-spectrum therapy was seldom adopted, and vaccination follow-up was limited. These findings highlight persistent stewardship and prevention gaps in IPD care. Embedding automated vaccine prompts and structured antimicrobial review may enhance both therapeutic precision and long-term disease prevention.

B111

Old Habits Test Hard: A Decade of Pneumococcal Urinary Antigen Use in Clinical Practice

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Abstract

Background

Pneumococcal urinary antigen (PUA) testing has long been used to support the diagnosis of community-acquired pneumonia (CAP), yet its clinical value has become increasingly uncertain. International guidelines have progressively reduced its recommended use, prompting reassessment of local practice.

Objectives

To evaluate PUA test utilisation, diagnostic yield, and influence on clinical management in a tertiary hospital over a ten-year period.

Methods

A retrospective review was conducted of all PUA tests performed between 2014 and 2023. Laboratory data were linked to clinical records to assess test positivity, antimicrobial prescribing decisions, and pneumococcal vaccination documentation at discharge. Hospital discharge coding identified tests performed in patients without a CAP diagnosis to estimate inappropriate use.

Results

A total of 7,178 tests were performed, with a positivity rate of 7.6%. Forty-two percent were ordered in patients without a CAP diagnosis. Among positive results, antimicrobial de-escalation occurred in only 13%, while vaccination was recommended at discharge in 14%. Estimated resource costs associated with non-indicated testing exceeded $17,000 annually.

Conclusions

PUA testing demonstrated low yield and limited impact on antimicrobial or vaccination practices, indicating poor alignment between diagnostic activity and actionable outcomes. Restricting testing to defined high-acuity or high-risk populations, in line with updated ATS/IDSA guidance, would strengthen diagnostic stewardship and optimise resource use.

B112

microSENSE: towards a rapid and automated biosensor for zoonotic pathogens.

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Abstract

Anthrax, caused by *Bacillus anthracis*, is a globally prevalent zoonotic disease with severe impacts on both humans and animals (Tyśkiewicz et al., 2023). Its spores are highly resistant to environmental stressors, making the detection challenging and posing a serious bioterrorism threat (Zasada, 2020). Current detection methods are limited by low sensitivity, matrix inhibition in environmental samples (Railean et al., 2024), and genetic similarity within the *Bacillus cereus* group (Cheun et al., 2003), underscoring the need for a rapid and field-deployable detection system.

We introduce a novel microwave-based approach that enables ultra-fast spore disruption and efficient DNA release, accelerating sample preparation time. Leveraging the specificity of DNA-based diagnostics, we aim to employ pathogen-specific DNA probes to differentiate *B. anthracis* from other members of the *B. cereus* group. The simplicity and scalability of microwave electronics enable easy miniaturisation, ideal for portable, on-site diagnostics. Furthermore, to enhance sensitivity, particularly in low-yield environmental samples, we integrate microwave-assisted isothermal amplification (Yoshimura et al., 2015), capitalising on thermal effects to streamline DNA processing.

The resulting system, microSENSE, is an 'all-in-one', automated biosensor capable of performing lysis, amplification, and fluorescent detection in an estimated time of one hour. Designed for point-of-care use, it supports rapid diagnostics in resource-limited and high-risk settings. Beyond anthrax, microSENSE holds potential for detecting other sporulating zoonotic pathogens, advancing infectious disease surveillance and global health security.

B113

Integrating Non-Culture Assays and Genomic Analysis for Invasive Aspergillosis Diagnostics: A Prospective, One-Year Study in a Resource-Limited Setting, Peshawar, Pakistan

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Abstract

Background: Invasive Aspergillosis (IA) remains a leading cause of morbidity and mortality in immunocompromised patients globally, with diagnosis hindered in Low- and Middle-Income Countries by limited culture facilities and high costs. We aimed to assess the diagnostic accuracy and comparative cost-utility of rapid antigen/molecular assays, including a targeted genomic analysis, to develop a sustainable, up-to-date diagnostic strategy in Peshawar.

Methods: This prospective, one-year (Jan–Dec 2025), observational study at a large tertiary hospital in Peshawar, Pakistan, enrolled 485 consecutive high-risk patients suspected of IA. All subjects were tested using serum Galactomannan (GM), plasma Beta-D-glucan (BDG), and an Aspergillus Lateral Flow Assay (LFA). Crucially, a subset of 120 bronchoalveolar lavage (BAL) and tissue samples underwent a targeted **qPCR and sequencing analysis (genomic analysis)** for *Aspergillus* species identification and detection of azole resistance markers. Accuracy metrics and cost-per-result were compared.

Results: Of the 485 patients, 62 (12.8%) met the criteria for IA. GM showed the highest individual sensitivity (79%). The LFA was the most cost-effective at 12$, achieving high specificity (97.7%) but lower sensitivity (61.3%). The targeted **genomic analysis** demonstrated 95.5% concordance with culture and provided actionable genotypic resistance information in 15 cases, offering the highest diagnostic certainty despite the initial cost.

Conclusion: Integrating cost-effective rapid diagnostics (LFA) with targeted **genomic confirmation** for high-certainty cases offers an optimal, multi-tiered diagnostic approach for IA in resource-constrained settings like Peshawar. While LFA provides rapid triage, genomic analysis is essential for definitive species identification and informing antifungal stewardship in the face of emerging resistance.

B114

Investigating the repurposing of metal-based photosensitizer compounds as antimicrobials.

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Abstract

Rising levels of antimicrobial resistance pose a significant threat to human health. With this ever-increasing burden, the need to uncover novel antibiotic agents becomes increasingly critical. The urgency of this need is enhanced by the void within antibiotic discovery, with the identification of *de novo* antibiotics declining with time. One strategy that holds promise is the potential for drug repurposing, whereby compounds originally intended for non-antimicrobial use can be repurposed for such gain. This approach removes the need for target-to-drug antibiotic discovery, beneficial due to the multiple challenges surrounding this. In the present work, we have identified a panel of metal-based photosensitizer compounds with *in vitro* bactericidal and bacteriostatic activity without light activation. These compounds have been repurposed from their originally intended use as novel chemotherapeutic agents. Compounds of this nature often take a multi-target approach to bacterial killing, reducing opportunity for future resistance development and offering potential for a more long-term solution to AMR. Multiple complexes demonstrated activity against the three *Mycobacterium abscessus* subspecies, *Mycobacterium bovis* BCG and/or ESKAPE pathogens, revealing five hit structures. Following chemical synthesis of these lead structures, the cytotoxicity against multiple cell lines was assessed using MTT assays and Annexin V/propidium iodide flow cytometry. Their toxicity thresholds against both bacterial species and mammalian cells were combined to assess the clinical utility of each compound. This work highlights scope for the development of an optimised metal complex that provides antimicrobial activity without off-target cytotoxicity.

B115

Distinct Functional Roles of *Pseudomonas aeruginosa* Extracellular Vesicles from Planktonic and Pellicle Growth

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Abstract

Extracellular vesicles (EVs) produced by *Pseudomonas aeruginosa* are known to contribute to bacterial communication, biofilm development, and host–pathogen interactions. However, the extent to which growth conditions influence the properties of these vesicles is not well understood.

Our study compared bacterial extracellular vesicles (BEVs) from *P. aeruginosa* PAO1 grown as planktonic cultures and air-liquid interface pellicles. BEVs were isolated using a modified method by Dauros Singorenko *et al.* (2017), and characterised using flow cytometry, NanoSight, SDS-PAGE, and 2D SDS-PAGE. Functional assays assessed their influence on biofilm biomass and A549 lung epithelial cellular responses. Lipopolysaccharide (LPS) controls were included.

SDS and 2D SDS PAGE analysis showed distinct protein profile differences between planktonic and pellicle BEVs. Functionally, pellicle-derived BEVs significantly increased biofilm formation ($p < 0.01$), whereas planktonic BEVs had no effect. In epithelial cell assays, planktonic BEVs enhanced metabolic activity after 24 hours ($p < 0.0001$), while pellicle BEVs reduced activity at both 24 ($p < 0.05$) and 72 hours ($p < 0.001$).

In conclusion, BEVs from different growth modes modulate biofilm dynamics and host cell responses in distinct ways, although the overall effects were modest. We will present the results of our mass spectrometry analysis of BEV proteins alongside the results of our BEV RNA sequencing from these two different growth states with the aim of elucidating potential mechanisms underpinning their role in pathogenesis and identifying potential therapeutic targets.

B116

From Culture to Code: Transforming Sepsis Diagnostics with Whole Genome Sequencing

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Abstract

Multidrug-resistant sepsis poses a major clinical challenge worldwide, particularly in critical care settings where delayed or inaccurate diagnosis can lead to inappropriate therapy and poor outcomes. Conventional diagnostic tools such as blood cultures and targeted PCR are limited by low sensitivity, narrow detection spectra, and prolonged turnaround times. This review explores the clinical potential of WGS as a comprehensive and culture-independent diagnostic solution for MDR sepsis. WGS enables high-resolution pathogen identification, antimicrobial resistance profiling, and strain typing from both culture-based and direct-from-sample approaches. It has demonstrated value in detecting fastidious, rare, or polymicrobial infections, supporting real-time therapeutic decisions, and facilitating antibiotic de-escalation. Clinical studies from diverse settings have shown that WGS can reduce diagnostic delays, improve treatment precision, and enhance antimicrobial stewardship.

To support clinical adoption, we outline a stepwise integration framework addressing workflow optimization, infrastructure development, bioinformatics capacity, staff training, quality assurance, and inter-institutional collaboration. We also highlight barriers including cost, data complexity, and regulatory ambiguity, while advocating for policy support, capacity building, and sustainable funding mechanisms. WGS has demonstrated clinical utility in various aspects of infectious disease diagnosis, including outbreak investigation, pathogen evolution and epidemiology, and antimicrobial resistance surveillance**.** Emerging tools such as portable sequencers and AI-driven analysis are further expanding the accessibility and impact of WGS at the bedside. Beyond individual diagnosis, WGS contributes to AMR surveillance and outbreak tracking, aligning with national and global public health strategies. With strategic investment, regulatory clarity, WGS transition from a technology to a clinical mainstay in the early, precise diagnostics.

B117

Strengthening Diagnostic Laboratory Infrastructure to Combat Antimicrobial Resistance in Rural Ghana: Challenges and Opportunities

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Abstract

Antimicrobial resistance (AMR) among bacterial pathogens is a major public health concern in Ghana, particularly in rural areas with limited diagnostic capacity and healthcare infrastructure. Empirical antibiotic use in these settings contributes to the spread of resistant strains. Strengthening AMR surveillance and diagnostics in such regions aligns with one of the five pillars of the WHO Global Action Plan on AMR. A proposed framework emphasizes (1) Infrastructure Development—equipping laboratories with essential bacteriology tools; (2) Capacity Building—training laboratory and healthcare personnel in diagnostics and antimicrobial stewardship; and (3) Sustainable Implementation—ensuring long-term integration into national health systems.

We report on the establishment of ongoing AMR surveillance in three rural districts: Agogo (2019), Assin Fosu (2020), and Agroyesum (2023). Each site houses a microbiology laboratory within the district hospital, equipped and staffed per WHO standards. Across these sites, 4,260 clinical samples (Agogo, 1,718; Assin Fosu, 2,074; Agroyesum, 470) comprising blood, urine, wound, and stool were analyzed. The predominant pathogens included *Escherichia coli*, *Klebsiella spp.*, *Enterobacter spp.*, *Staphylococcus aureus*, and *Acinetobacter spp.*Laboratory data have enhanced clinical decision-making through improved bacterial identification and antibiotic susceptibility testing, facilitating targeted therapy and informed empirical treatment. Regular AMR reports are shared with clinicians, and data are uploaded to WHONET under GLASS guidelines.

We highlight the progress, impact, and prospects of AMR surveillance in rural Ghana, as well as challenges such as logistical limitations, clinician uptake of culture-based diagnostics, and strategies for long-term sustainability.

B118

High-throughput chemical genomic screening analysis of 1,465 Klebsiella pneumoniae clinical isolates

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Abstract

High-throughput chemical genomics enables the analysis of strain library phenotypes under specific chemical and environmental conditions to uncover gene functions. This approach supports the mapping of biological pathways and the identification of potential drug targets. Such datasets have driven numerous hypothesis-based studies with important implications for antimicrobial resistance and clinical outcomes. High-throughput phenotypic profiling also enriches microbial sequence data with functional annotations, benefiting the wider scientific community.

In this study, we combine high-throughput phenotypic assays, whole-genome sequencing and statistical machine learning to predict a range of growth-related traits in 1,465 clinical isolates of *Klebsiella pneumoniae*, including both hypervirulent (hvKP) and multidrug-resistant (MDR-KP) variants. We comprehensively profiled growth rates across 250 conditions, including subinhibitory antibiotic concentrations, diverse chemical stressors, varying media compositions and multiple pH levels. Our findings provide new insights into the fitness landscape that underlies complex growth phenotypes in *Klebsiella pneumoniae*.

B119

Describing the Epidemiology and Microbiology of *Escherichia albertii* in England, 2014 – 2025.

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Abstract

Background: *Escherichia albertii* is an emerging, understudied enteric pathogen whose clinical impact is obscured by frequent misidentification and limited surveillance.

Aims: To characterise the epidemiology, antimicrobial-resistance (AMR) profile, and population structure of *E. albertii* isolates reported to the UK Health Security Agency (UKHSA) in England between 2014 and 2025.

Methods: A dataset of 159 human isolates was analysed using whole-genome sequencing, phylogenetic typing and linked epidemiological metadata (age, sex, travel, clinical presentation). Temporal trends, seasonal distribution, geographic spread, and AMR determinants (including *gyrA* mutations) were examined.

Results: Annual case numbers fluctuated with periods of low-incidence interspersed by peaks in 2019 (23 cases) and 2024 (25 cases). Cases peaked in July, with the highest incidence in children <10 years (48 cases) and a male predominance (88 M vs 70 F). Travel history was unknown for most isolates; when reported, Asia accounted for 67% of recent travel (23/34). Diarrhoea was the primary symptom (70% of 113 cases). Fifty-four isolates harboured *gyrA* mutations, and 42/159 were multidrug-resistant (MDR). Since the COVID-19 pandemic, an MDR sequence type, ST5390, has emerged, predominantly among adult men who have sex with men (MSM). The MDR phenotype of this ST5390 MSM cluster likely reflects selective pressure from higher antibiotic usage within that community. Most isolates carried two-three plasmids, promting future work on virulence-gene associations.

Conclusions: *E. albertii* shows increasing temporal prevalence, seasonal peaks, and a concerning MDR ST5390 cluster linked to MSM transmission. Integrated genomic-epidemiological surveillance is essential to monitor virulence, AMR, and transmission dynamics, guiding public-health interventions.

B120

Exploring Salmonella Infections in Young Children: Insights from 20 Years of Surveillance in England and Wales

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Abstract

Infections caused by *Salmonella* pose a greater threat to vulnerable populations, such as young children, the elderly and the immune-compromised. The aim of this study was to investigate the differences in prevalence of *Salmonella* serovars between patients under and over five years old.

From 2004 to 2023, isolates of *Salmonella* referred to the UK Health protection Agency for serotyping were analysed, in conjunction with demographic data including age, sex, and area of residence.

The total number of *Salmonella* isolates was 216,236, of which 29,331 (13.6%) were from pre-school children aged under five years old. Overall, notifications in this age group have declined at a similar rate to individuals aged five and over, although the impact of the Coronavirus disease pandemic was less pronounced. The highest incidence was in 1–2-year-olds, whereas the lowest was in 4-years old children. Of the top 20 serovars, 18/20 matched between the two age groups, with *S.* Enteritidis being the most common in both cases. *S.* Poona and *S.* Weltevreden were in the top 20 for pre-school children, but not the older age group. Trend analyses of case numbers of *S.* Typhimurium in the pre-school group, revealed increases in 2009 and 2011, linked to outbreaks associated with contaminated reptiles or reptile feeder mice.

Salmonellosis trends in pre-school children reflect those observed in the general population. Of the children aged under five, those aged 1-2 years old were most at risk. Outbreak investigations revealed that exposure to reptiles was a heightened risk for pre-school children.

B121

Determining The Relationship Between Thermotolerant Coliforms and Sanitary Survey Contamination Risk Factors Within TA Somba, Malawi

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Abstract

5.57 million people lack access to basic water services and clean water in Malawi, causing significant mortality. Sub-Saharan Africa shows the least improvement in access to clean water globally, with a 40% increase in the number of people lacking access to safe water since 2000. One cost-effective strategy Malawi has deployed in order to reach the UN SDG6 is sanitary inspection surveys (SIS). Questionnaires categorised water points by contamination risk based on theoretical risk factors, allowing resources to be better focused.

This quantitative study used a retrospective case-study approach to explore whether the presence of risk factors recorded in the SIS had any correlational relationship to thermotolerant coliform (TTC) colony counts, a proxy indicator for faecal contamination.

Results identified that only 3 of the 21 risk factors analysed have statistically significant positive correlational relationships to increasing TTC colony counts. These risk factors were:

1. Source of contamination within 30m of the waterpoint
2. Well apron cracked or damaged
3. Spilt water collecting in the well apron

Results were in line with existing literature that SISs are unreliable at risk categorisation, as different risk factors have equal weighting on the risk factor category, but have varying associations with TTCs colony counts in real life. Unexpectedly, results identified the lack of a fence around a waterpoint to prevent animal access was not associated with increased TTCs, suggesting deeper analysis is needed to investigate the relation between animals and agriculture on TTCs specifically, as this risk factor encompasses multiple potential factors.

B123

RAFT Buffer: Rapid, Field-Deployable One-Step DNA Extraction and Preservation from Stool and Sewage for Molecular Diagnostics

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Abstract

Molecular diagnostics for enteric pathogens in low-resource settings are hindered by complex sample preparation and cold-chain requirements. Conventional DNA extraction methods are time-intensive, rely on specialized equipment and trained personnel, and require refrigerated reagents, limiting rapid field deployment. To address these limitations, we developed RAFT Buffer, a field-deployable, PCR-compatible solution for rapid, one-step DNA extraction and nucleic acid preservation from stool and sewage samples without the need for equipment or refrigeration. Samples (50 µL) were mixed with 450 µL of RAFT Buffer and incubated for 15 minutes, after which DNA yield, purity (A260/280 and A260/230), and PCR amplification performance were evaluated. Across *Enterotoxigenic Escherichia coli* (ETEC) cultures, human stool, and sewage, RAFT Buffer consistently produced amplifiable 570 bp 16S rDNA fragments with high analytical sensitivity (OD_{600} = 0.17) and specificity. Accelerated stability tests at 21 °C and 37 °C over five days confirmed robust performance under ambient storage, eliminating cold-chain requirements. PCR validation demonstrated reliable amplification across sample types, temperatures, and timepoints, highlighting resilience under simulated field conditions. By replacing multi-step workflows and removing equipment and cold-storage constraints, RAFT Buffer streamlines molecular diagnostics in resource-limited environments. The method also supports local reagent production, reduces per-test costs, mitigates supply chain vulnerabilities, and promotes equitable access to enteric disease surveillance. RAFT Buffer provides a practical, scalable, and field-ready solution for rapid pathogen detection from stool and sewage, enabling timely molecular diagnostics in low-resource and remote settings.

B124

Experimental evolution of antibiotic resistance in the ex vivo pig lung biofilm model.

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Abstract

Biofilms occurs in 65% of all infections and 80% of chronic infections with major implications for AMR. Understanding the role biofilms play in AMR evolution is important to fully assess AMR acquisition. Here, we performed experimental AMR evolution in the *ex vivo* pig lung model of cystic fibrosis lung biofilm (EVPL) and compared the results with those from a selection experiment using planktonic cultures in synthetic CF sputum media, to identify the adaptive landscape of meropenem resistance in more physiologically relevant conditions.

Pseudomonas aeruginosa was selected for resistance to meropenem in the EVPL using an evolutionary ramp approach. We showed that biofilm on an inoculated piece of porcine bronchiole can seed a new biofilm on an uninoculated piece. Serial passage via colonisation of new tissue pieces was continued for 10 days. Evolved clones were whole genome sequenced and mutations identified bioinformatically and collateral susceptibility to other antibiotics and comparative fitness were assessed.

Meropenem selection began at 0.25x the MIC in lung homogenate media (0.0625mg/ml) and reached a final meropenem concentration of 8mg/ml (128xMIC). Clones from six independent populations were isolated at days 5 and day 10. These clones were sequenced with Illumina short-read sequencing and the impact on these mutations on fitness was assess comparatively.

Using the EVPL model for meropenem selection highlighted differences from previous planktonic selection experiments. 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.

B125

An *in-vitro* model of late-stage plague infection for the investigation of immune modulating drugs

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Abstract

Plague is an infectious disease caused by the Gram-negative bacterium *Yersinia pestis.* Plague is classified as a re-emerging disease, causing periodic outbreaks in endemic countries. There is currently no licensed vaccine and, whilst antibiotics are often effective, antibiotic-resistant strains remain a concern (Lei and Kumar 2022). Late-stage plague infection can result in an excessive and detrimental delayed inflammatory response. Immune-modulatory drugs could provide a novel therapeutic approach.

We have developed a simplistic *in-vitro* model of septicaemic plague infection using THP-1 monocyte-like cells. Cells were stimulated with formalin-inactivated *Y. pestis*. Pro-inflammatory cytokine release (CCL2, IL-18, IL-1β, IL-6, IL-8 and TNF-α) was measured using automated ELISA. 12 immune-modulating drugs were then added to the cell culture at different pharmacodynamically relevant concentrations before and after stimulation with inactivated *Y. pestis*.

We show that the corticosteroids, dexamethasone and prednisolone, and MCC950 (an inflammasome inhibitor) were the most effective at reducing inflammatory signals. Niclosamide and nitazoxanide, both anti-helminthic drugs, also markedly reduced some signals. This study demonstrated the potential utility of these drugs in the treatment of plague. To the best of our knowledge there are no published studies regarding the efficacy of MCC950, niclosamide or nitazoxanide in *Y. pestis* infection. These drugs could represent a novel treatment adjunct for plague.

Lei, C., and S. Kumar. 2022. 'Yersinia pestis antibiotic resistance: a systematic review', *Osong Public Health Res Perspect*, 13: 24-36.

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B126

Association between spirochaetal infection and neurodegenerative diseases - a systematic review of observational studies

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Abstract

Neurodegenerative diseases, including Alzheimer's and Parkinson's, are a growing global health concern. While age remains the primary risk factor, infectious agents have been proposed as contributors to disease onset or progression. Spirochaetal bacteria, such as *Treponema pallidum* (syphilis), *Borrelia burgdorferi* (Lyme disease), and *Leptospira* spp. (leptospirosis) are known to invade the central nervous system and may play a role in neurodegeneration. This poster presents a systematic review of observational evidence on the association between spirochaetal infections and neurodegenerative diseases.

The systematic search was performed within five databases: SCOPUS, EMBASE, PubMed (MEDLINE), Web of Science, and EBSCO (CINAHL), in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Registration no: CRD420251014990). Eligible studies involved adult human populations, and reported both infection and cognitive or neurodegenerative outcomes using standardised methods. Data were narratively synthesised due to heterogeneity in study design. Risk of bias was assessed using the Newcastle-Ottawa Scale.

Twenty-seven studies met the inclusion criteria: *T. pallidum* n = 13, *B. burgdorferi* n = 13, and *Leptospira* spp. n = 1. No eligible studies were found for *Brachyspira* spp., and studies involving *T. denticola* were excluded due to the confounding presence of periodontitis. Studies on syphilis and leptospirosis consistently reported cognitive impairment and increased dementia risk. In contrast, findings for Lyme disease were heterogeneous; the majority found no long-term cognitive effects, while others reported persistent cognitive symptoms or increased Alzheimer's risk. Future research should seek to investigate the molecular and physiological basis of this association.

B127

Determining the mechanism of killing of lectin-like protein antibiotics

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Abstract

In recent years there has been a global increase of antibiotic resistant infections. *Pseudomonas aeruginosa* is an important cause of hospital acquired infections that are associated with high levels of mortality. *P. aeruginosa* infections are becoming increasingly difficult to treat, with between 18 and 25% of clinical isolates showing multidrug resistance and isolated found across the globe which are resistant to all or nearly all available antibiotics.

Bacteriocins, antimicrobial proteins deployed by bacteria for competition and colonisation, offer a potential tool to combat antibiotic resistance. The narrow spectrum of bacteriocins, which generally target bacteria closely related to the producing strain, could enable targeting of specific pathogenic bacteria without causing damage to the wider microbiome. There has been a range of bacteriocins discovered against *P. aeruginosa,* known as pyocins, with lectin-like pyocin L1 showing efficacy in infection models. However, we currently lack understanding of the mechanism of action of pyocin L1, which limits its development as a potential antibiotic therapy.

Our current aim is to identify the molecular target of pyocin L1 in *P. aeruginosa* using a range of genomic, biochemical and biophysical analyses, We are also further testing the activity of pyocin L1 against *P aeruginosa* biofilms and determining its ability to enhance the activity of other anti-pseudomonal antibiotics.

B128

Lost in summary: Communication of Clostridiodes difficile infection to General Practitioners in acute hospital discharge summaries.

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Abstract

Background: Effective communication of *Clostridioides difficile* infection (CDI) to General Practitioners is vital for safe continuity of patient care and antimicrobial stewardship. Deficiencies in discharge documentation incurs increased risk of inappropriate prescribing, missed follow-up and potential community transmission. We aimed to evaluate the completeness and accuracy of discharge communication regarding CDI from secondary to primary care, with a focus on identifying gaps and informing targeted interventions.

Methods: In 2024, 149 patients had laboratory-confirmed CDI in Beaumont Hospital. Admission dates and anti-CDI treatment data were extracted from surveillance records. Electronic patient discharge summaries were reviewed and correlated with laboratory information system records. Fourteen cases managed by GPs or in long-term care settings were excluded. Data on the remaining 135 hospitalised patients were analysed.

Results: Of 135 patients, 16.3% (n=22) had no discharge summary completed. Of these, 6 died during admission. The remaining 16 were discharged without documented correspondence to their GP. Among the 113 available discharge summaries, 23 (20.4%) failed to document CDI during admission. These included 16 medical and seven neurosurgical patients. Four medical patients died during admission from complications unrelated to CDI, and their discharge summary included limited clinical details with date and cause of death.

Conclusions: This study identified a significant communication gap; 16.3% patients had no discharge communication and 20.4% of completed discharge summaries omitted CDI diagnosis and management. This has implications for patient safety, infection control, and continuity of care. Proposed interventions include targeted clinician education, specifically in neurosurgery and in the medical directorate with re-audit.

B130

Salmocins for the treatment of antibiotic-resistant Salmonella infections

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Abstract

Salmocins for the treatment of antibiotic-resistant *Salmonella* infections

Awang Najmuldeen, Yasmin McLaughlin, Elizabeth Cairns, Helina Marshall, Daniel Walker

Antimicrobial resistance (AMR) is increasing globally and is now recognised as a major threat to human and animal health. Although many *Salmonella* infections in humans are self-limiting, a significant proportion progress to severe disease that requires antibiotic therapy. These cases are particularly problematic in vulnerable patients and in invasive non-typhoidal *Salmonella* disease. In recent years, resistance to frontline antibiotics has risen sharply across both clinical and agricultural settings, reducing effective treatment options and complicating disease management.

Salmocins are protein antibiotics derived from bacteriocins that naturally and selectively target *Salmonella* species. Through species-specific mechanisms of action, salmocins target pathogenic *Salmonella* while preserving commensal bacterial communities. This narrow-spectrum approach provides important advantages, including reduced selection pressure on non-target organisms and a lower likelihood of spreading AMR determinants within microbial populations.

We present recent data demonstrating the potency, selectivity, and efficacy of salmocins against *Salmonella isolates, including drug-resistant strains*. These findings support the development of salmocins as targeted alternatives to broad-spectrum antibiotics for treating severe *Salmonella* infections and for use in agricultural disease control.

B133

Approach to merge individual RAA assays into a multiplex RAA design for simultaneous detection of multiple Schistosoma specie

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Abstract

Isothermal amplification methods such as LAMP and recombinase-based amplification (RAA/RPA) are increasingly valuable tools for point-of-care and field diagnostics. They offer rapid results without the need for thermocyclers, making them suitable for decentralized testing setups.

However, a major bottleneck remains: multiplexed isothermal detection.

While numerous single-plex isothermal assays have been published, strategies to combine them into reliable duplex or multiplex assays are limited.

Here, we set out to design a duplex RAA assay for simultaneous detection of *Schistosoma mansoni* and *S. haematobium*. Our approach builds on validated single-plex assays from the literature, reducing development time while focusing on sensitivity, specificity, and compatibility with portable instrumentation.

Because we intend to apply the developed duplex assay for environmental monitoring as well as clinical diagnostics, we prioritized assay performance over other parameters such as assay speed.

B134

From Monoculture to Microbiome: How species interactions influence the evolution of meropenem resistance in *Pseudomonas aeruginosa*.

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Abstract

The emergence and spread of antimicrobial resistance poses a significant risk to human health and the ability to successfully treat bacterial infections. Conventional evolution experiments, in which single-species cultures of bacterial pathogens are exposed to antibiotics in nutrient-rich laboratory media, have provided fundamental insights into antibiotic resistance mechanisms. However, these laboratory cultures fall short of representing the complexity of the biological environments where infections occur. Bacteria rarely live in isolation, but often exist as part of larger communities where both competitive and cooperative interactions may take place. During the process of infection, bacterial pathogens can interact with a variety of other microbial species, and such interactions may influence how they respond to antibiotic treatment. Our aim in this project was to investigate how the presence of other microbial species impacts the evolution of antibiotic resistance in a bacterial pathogen. The opportunistic pathogen *Pseudomonas aeruginosa* in the context of the respiratory microbiome was used as the model system to explore this question in. We conducted an adaptive laboratory evolution experiment to investigate the impact of species interactions on the evolution of meropenem resistance in *P. aeruginosa*. Twenty independent lines of *P. aeruginosa* were passaged through the spent media of six different respiratory microbes under increasing concentrations of meropenem. Genome sequencing in parallel with a range of phenotyping assays will be carried out in order to understand when and how species interactions can impact meropenem resistance evolvability and the evolutionary trajectories by which resistance is achieved in *P. aeruginosa*.

B135

Characterizing Host-Pathogen Interactions Involved in *Listeria monocytogenes* Invasion of the Brain

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Abstract

Listeria monocytogenes (*Lm*) is a leading cause of foodborne illness-related mortality in the UK and the US. During infection, *Lm* replicates within human cells and spreads directly from cell to cell, ultimately enabling infection of vital organs such as the brain. A key mechanism underlying this dissemination is actin-based motility, driven by the bacterial surface protein ActA. ActA nucleates polymerization of host actin to form 'comet tail' structures that propel bacteria through the host cytosol and into adjacent cells. However, the molecular interactions between host and bacterial factors supporting this motility remain incompletely understood. We recently identified the host protein EPS8 as a component of *Lm*-induced actin comet tails. However, the role of EPS8 and the mechanism by which EPS8 is recruited to these structures is unclear. To investigate the potential role of ActA in recruiting EPS8 to cytosolic *Lm*, we generated an *actA* deletion mutant of *Lm* expressing blue fluorescent protein (*Lm* Δ*actA*-BFP*)*. We then used the *Lm* Δ*actA*-BFP mutant to infect HeLa human epithelial cells expressing EPS8 fused with an mCherry fluorescent tag. Using spinning-disk confocal microscopy, we observed that *Lm* Δ*actA*-BFP lost co-localization with EPS8 compared to wild-type *Lm*, indicating that ActA plays a critical role in recruiting EPS8 to *Lm* during infection. Further studies will focus on determining whether EPS8 interacts directly with ActA, or indirectly via ActA-associated factors, and identifying the domains of EPS8 required to promote *Lm* actin-based motility. Together, these findings may reveal novel targets for therapeutic intervention against *Lm* infection.

B136

The role of the nematode worm *Caenorhabditis elegans* as reservoir for *Yersinia pseudotuberculosis* in the soil environment

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, Luke Shipley, Hannah Ford, Steve Atkinson

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Abstract

The enteropathogen *Yersinia pseudotuberculosis* causes gastric infections and is found free living in soil environments. At the DNA level, *Y. pseudotuberculosis* is extremely closely related (98%) to the highly virulent fulminant pathogen *Yersinia pestis*, the cause of plague (Black Death). Given the dangers of working with *Y. pestis*, *Y. pseudotuberculosis* acts as a model when considering plague microbiology. Both *Y. pseudotuberculosis* and *Y. pestis* are known to form biofilms on the surface of *Caenorhabditis elegans* nematodes. We hypothesise that *C. elegans* infected with *Y. pseudotuberculosis* biofilm might act as a reservoir for transmission of *Yersinia spp*. in soil.

We therefore infected *C. elegans* with *Y. pseudotuberculosis* to allow biofilms to form on their surface. After 48 hours at 22°C, infected *C. elegans* were transferred into sterile model soils. After 3 days, soils were plated on selective agar to recover surviving worms and examine for biofilms. To verify whether the surviving *C. elegans* or infected soils contained *Y. pseudotuberculosis,* both were grown on *Yersinia spp*. selective agar. In addition, PCR targeting *yopJ* as a marker for the presence of the *Yersinia* virulence plasmid was also performed.

For soil and *C. elegans*, colonies growing on culture media were positive for *yopJ*. However, PCR of soil supernatant remain negative, probably due to the presence of PCR inhibitors in soil. Detection of *Y. pseudotuberculosis* in soil and recovered from *C. elegans* suggests that nematode worms have the potential to act as a reservoir for *Yersinia spp* and by extension *Y. pestis*.

B137

Selection and characterisation of a collection of lab-derived and clinical antibiotic-tolerant Escherichia coli strains: an essential resource

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, Joanne Hobbs

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Abstract

Antibiotic tolerance is the ability of a bacterial population to survive exposure to a bactericidal antibiotic for longer than a susceptible strain, without exhibiting resistance. Despite links to recalcitrant infections and treatment failures, tolerance remains poorly understood. Therefore, a panel of mutationally diverse tolerant *Escherichia coli* strains was created, with which the mechanisms and clinical significance of tolerance could be studied. Three approaches were used to assemble this panel: 1) reported tolerance mutations were introduced into a model non-tolerant strain using allelic exchange; 2) tolerant strains were selected following *in vitro* evolution, where *E. coli* was exposed to intermittent antibiotic treatment; and 3) clinical isolates and engineered strains with point mutations in tolerance-associated genes were acquired from collaborators. The resistance, tolerance and growth profiles of these strains were determined, and evolved strains were sequenced to uncover their tolerance mutations. The *in vivo* significance of tolerance on antibiotic treatment outcomes was assessed using *Galleria mellonella*. Overall, a panel of >30 strains with diverse tolerance mutations was assembled and characterised, and sequencing of evolved strains revealed novel tolerance mutations in genes associated with metabolism and growth. Infection of *G. mellonella* with tolerant *E. coli* strains led to reduced survival and increased bacterial burden following antibiotic treatment. This panel of tolerant strains represents an essential resource for the tolerance research community and will be used to further study the mechanisms and significance of tolerance. The finding that tolerance negatively impacts antibiotic treatment in an animal model demonstrates the clinical relevance of tolerance.

B138

Understanding how Pseudomonas aeruginosa biofilms respond to ciprofloxacin exposure using transposon directed insertion-site sequencing (TraDIS)

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Abstract

Pseudomonas aeruginosa (PA) is an opportunistic pathogen that cause severe infections in immunocompromised individuals, such as patients with cystic fibrosis. Ciprofloxacin, a fluoroquinolone antibiotic, is used extensively to treat PA infections but has significantly reduced effectiveness due to biofilm. Although many genes associated with biofilm formation or ciprofloxacin resistance have been characterised, how PA maintains biofilm when challenged with ciprofloxacin is not fully understood. In this regard, we employed a transposon-directed insertion-site sequencing (TraDIS-*Xpress*) approach to assay the impact of gene disruptions or altered gene expression across the entire genome on survival of biofilms exposed to different concentrations of ciprofloxacin. Gene fitness was analysed by comparing biofilm samples to planktonic controls at different stages of biofilm formation. Genes involved in c-di-GMP signalling, quorum sensing, as well as some global regulators were potential key determinants for biofilm formation, validating the method. Beyond these common regulators, under ciprofloxacin exposure, flagellar function and exopolysaccharide production were essential for initial biofilm formation. Moreover, mutants in LPS gene clusters and the peptidoglycan recycling genes had reduced fitness in biofilm, indicating that the O-antigen structure of LPS and cell wall integrity are crucial for biofilm survival in the presence of ciprofloxacin. Several putative genes of unknown function were found to be specifically important for biofilm formation under ciprofloxacin stress and further characterisation of these genes is currently underway. Taken together, this study provided a genome-wide insight into the interplay between biofilm formation and ciprofloxacin treatment and identified potential therapeutic targets for antibiofilm drug development.

B139

Combatting antimicrobial resistance: Small molecule demethylase inhibitors as precision antimicrobial and anti-virulence agents.

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Abstract

Antimicrobial resistance (AMR) is a growing global public health issue, and it is estimated that deaths associated with AMR infections will exceed 10 million by 2050. Methicillin-resistant Staphylococcus aureus (MRSA) remains a priority pathogen for the development of novel treatment strategies. Evolving virulence led mechanisms of resistance within microbial populations continually render current therapeutics infective and threaten a global return to the pre-antibiotic era. Small-molecule inhibitors (SMIs) designed against epigenetic bacterial processes, including methylation, represents an innovative approach to combatting AMR by modulating several key bacterial virulence traits. Preliminary antimicrobial susceptibility screening identified several lead candidate demethylase SMIs which demonstrated activity at <3.125µM against several clinically relevant species of MRSA. Anti-biofilm activity of one lead compound exhibited >90% eradication of established 24 and 48 h biofilms, in addition to inhibiting metabolic rate of 24 h biofilms by >48%. A >56% increase in generation of intracellular reactive oxygen species was triggered following 1 h SMI exposure at sub-lethal concentrations. Inhibition in bacterial mediated haemolysis was observed by 23% in cells exposed to sub-inhibitory concentrations compared against untreated populations. RNA sequencing analysis revealed regulatory changes in several key ATP synthesis and transmembrane metal transport system proteins. Potentiometric fluorescent probe assays exhibited SMI mediated disruption of bacterial membrane potential at sub-lethal concentrations. Research is now focused on validation of SMI bacterial target sites and identifying global proteomic changes to further elucidate the role of methylation via MALDI-MS and global protein methylation profiling.

B140

Time-equivalent experimental evolution of putatively persistent neonate-colonising Enterobacteriaceae

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Abstract

Extended-Spectrum β-lactamase producing *Enterobacteriaceae* (ESBL-*E*) are an urgent threat to global health, with lower-middle income countries bearing the greatest burden. Neonates are a vulnerable demographic with life stage-specific factors that limit generalisations from adult or mixed-age studies. Emergent from the ProRIDE randomised placebo-controlled clinical trial, six ESBL-*E* pairs were suspected to have persistently colonised their respective neonates. However, two pairs exhibited estimated mutation rates (7 SNPs, 10.11 SNPs genome^{-1} year^{-1}) beyond current clonal thresholds (5.5 SNPs genome^{-1} year^{-1}).

Through a time-matched experimental evolution of ancestral isolates, we demonstrated that ESBL-*E* can accumulate 2 – 6 SNPs (2.89 – 8.67 SNPs genome^{-1} year^{-1}), corresponding to a mutation rate of 6.5075 x 10^{-10} to 26.1992 x 10^{-10} base^{-1} generation^{-1} (95% CI). We estimated that ancestral strains would require either a significantly higher mutation rate or 1.07 – 3.59x generations to mutate the maximum persister SNPs within 18 weeks, though the latter is achievable through daily passages. Observed mutation rates, target genes, morphologies and growth dynamics were consistent with other experimental evolutions, and no hypermutation-associated mutations were identified. Furthermore, the ESBL phenotype remained dominant in almost all ancestries despite no antibiotic selection pressure.

We concluded that clonality thresholds likely underestimate the evolutionary potential of ESBL-*E* during early infancy, whereas experimental evolution can effectively model the life stage. Although we deferred to a statistical explanation, a hypothetical biological difference in mutation rates between neonates and adults cannot be discounted.

B141

Assessing pulmonary outcomes in non-ABPA *Aspergillus fumigatus* colonisation in cystic fibrosis

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Abstract

Background: In cystic fibrosis (CF), allergic bronchopulmonary aspergillosis (ABPA) is a known contributor to lung function decline and an inflammatory phenotype. Yet, the impact of non-ABPA *Aspergillus fumigatus* (AF) colonisation in CF is not sufficiently characterised.

Aim: To evaluate the impact of non-ABPA on clinical measures in people with CF (pwCF), and to characterise the cytokine profiles associated with AF presence, using a gold-standard ex vivo cystic fibrosis lung model.

Method: A systematic review and meta-analysis of approximately 15 published studies is being performed to compare clinical outcomes (FEV1, BMI) between non-ABPA AF-colonised pwCF and those without AF colonisation. Data collection is expected to be completed by December 2025.

Next, we will inoculate an *ex vivo* pig lung model (EVPL) containing a synthetic cystic fibrosis media (SCFM) with a laboratory strain of A. fumigatus. Cytokine profiles will be quantified via an ELISA assay, and histological analyses will evaluate tissue integrity and the extent of hyphal penetration. Data collection will be completed in April 2026.

Expected results: We hypothesise that non-ABPA AF colonisation will be associated with a decline in pulmonary function. In the EVPL model, AF colonisation is expected to induce an inflammatory response; either self-limiting or progressive.

Significance: Results may inform the clinical significance of non-ABPA in pwCF and inform the early markers of inflammation. This may guide future clinical management strategies for pwCF.

B142

Development of a novel multiplex PCR for the simultaneous detection of Panton-Valentine leukocidin (PVL), toxic shock syndrome toxin 1 (TSST-1) and methicillin resistant Staphylococcus aureus (MRSA)

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Abstract

Staphylococcus aureus (S. aureus) causes a variety of human diseases, where several secreted virulence factors participate, including Toxic Shock Syndrome Toxin-1 (TSST-1) and Panton-Valentine leucocidin (PVL). Methicillin resistant *S. aureus* (MRSA) is harder to treat, associated with increased morbidity and mortality and is a particular concern within hospitals worldwide.

A multiplex real-time PCR assay for all three targets is currently not available. This could enable diagnostic microbiology laboratories to detect these toxins and antimicrobial resistance as part of their normal screening processes, reducing turnaround time on results and improving patient outcomes.

Primer design was performed for MRSA genes (*MecA* and *MecC*), PVL genes (*Luk-S PV*) and TSST-1 (*tst*) gene, and a *S. aureus* specific 16S to use as a negative control. Primers were designed using primer-Blast. Conservation of primer binding sites in phylogenetically distant strains of *S. aureus* was confirmed by multiple sequence alignments using multiple sequence comparison by log- expectation (MUSCLE).

Initial analysis of the primer designed was by end-point PCR and agarose gel PCR. Extraction of *S. aureus* isolates(NCTC 13435 PVL, 11963 MRSA (mecA) 12493, *S. aureus* 12973 TSST-1, NCTC) was performed using Instagene matrix, and the PCR was carried out using Immomix polymerase.

Results showed success in primers for *S. aureus* specific 16S, MRSA MecC gene, TST gene, work still needs to be carried out to ensure the success of PVL detection prior to real-time PCR multiplex analysis on the QuantStudio 5. These results will be completed and presented at the conference.

B143

Stringent response (Rel) mutations are clinically common, confer tolerance to antibiotics and macrophage killing, and result in worse treatment outcomes *in vivo*.

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Abstract

The stringent response is a stress response in bacteria mediated by guanosine tetra- and pentaphosphate ([p]ppGpp). In most bacteria, (p)ppGpp is both synthesised and hydrolysed by the enzyme Rel. Rel modulates the cellular concentration of (p)ppGpp, with elevated levels leading to slower growth. This slow growth causes antibiotic tolerance, whereby a bacterial population survives transient exposure to an otherwise lethal concentration of antibiotic without exhibiting resistance. We have previously shown that a Rel mutation identified in a clinical isolate of *Staphylococcus aureus* confers antibiotic tolerance. Here, we evaluate the ability of diverse Rel mutations from clinical isolates of *S. aureus* and *Enterococcus faecalis* to confer *in vitro* tolerance and their impact on *in vivo* antibiotic treatment outcomes. We introduced four previously reported *S. aureus* Rel mutations and seven new clinical *E. faecalis* Rel mutations into non-tolerant strains *via* allelic exchange. Growth curves and time-kills revealed that most of these mutations confer an extended lag time and/or increased doubling time) and antibiotic tolerance. Tolerant Rel mutants also exhibited tolerance to killing by macrophages. Finally, we investigated the impact of Rel-mediated *in vitro* tolerance on antibiotic treatment outcomes in *Galleria mellonella*. Treatment outcomes in larvae infected with tolerant Rel mutants were worse than those infected with wildtype (larval survival and bacterial burden). We conclude that Rel mutation is a clinically common cause of antibiotic tolerance with potential as a tolerance biomarker. Consistent with its association with persistent infections, Rel-mediated tolerance leads to worse treatment outcomes *in vivo*.

B144

Exploring the role of plastic degrading enzymes in pathogenesis and persistence

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Abstract

The identification and characterisation of numerous environmental microorganisms capable of breaking down plastics has prompted efforts to harness both these microbes and their associated plastic-degrading enzymes as bioremediation tools for mitigating plastic pollution. Yet, investigations into such biodegradative capabilities have largely excluded pathogenic species. This gap is striking given the pervasive presence of plastics in clinical environments and medical equipment, along with mounting evidence that microplastics can accumulate within human tissues. Should pathogenic bacteria encode functional plastic-degrading enzymes, their potential influence on microbial survival, adaptation, and behaviour within healthcare settings warrants serious consideration. We hypothesise that clinical bacterial isolates may be a previously unrecognised reservoir of plastic-degrading enzymes which could render plastic-containing medical devices susceptible to degradation and failure. This could also increase risk and virulence of nosocomial infection, should these pathogens utilise plastic to grow, accelerate colonisation, or shield from decontamination. In this work, we identify several clinical isolates that display the ability to degrade plastic. We subsequently examine the impact of this enzymatic capability across a range of *in vitro* and *in vivo* models revealing a direct link to pathogenicity and persistence. Given the key role of plastic in healthcare, screening nosocomial bacteria for plastic-degrading capacity should be an important future consideration.

B145

Lead optimisation and screening of novel antibiotics compounds to treat life-threatening antibiotic resistant bacterial infections

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Abstract

Antimicrobial resistance (AMR) is one of the world's leading causes of death. This 'silent pandemic' requires novel drug discovery to treat common infections which are becoming increasingly difficult to clear. This project will evaluate the antimicrobial activity of our novel anthraquinone derivatives, (conjugated with delocalised lipophilic cations [DLC] through an ester or amide bond) by investigating the mechanism of activity of these compounds against ESKAPE pathogens (highly virulent multi-drug-resistant bacterial pathogens that are a major cause of hospital-acquired infections).

We hypothesise that these cationic lipophilic hybrid molecules can target bacterial membranes directly or transport their drug cargo across membranes to target bacterial topoisomerases.

Novel anthraquinone-DLC conjugates **MK-7** and **MK-22** were synthesised by conjugation of TPP (triphenyl phosphonium cation) with an amino butanol to halogenated anthraquinone derivatives via an ester bond.

The MICs of **MK-7** and **MK-22** were evaluated against both methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* (MRSA & MSSA). Both compounds demonstrated antibacterial activity, exhibiting an **MIC of 2 μg/mL**. Control antibiotic gentamicin exhibited an MIC of 0.5 μg/mL, the comparable efficacy of **MK-7** and **MK-22** highlights their promising potential as effective alternatives to conventional antibiotics, particularly considering their novel chemical structures and potential to overcome existing resistance mechanisms.

Notably, both conjugates inhibited the growth of planktonic MRSA and MSSA. The next steps will be to screen their efficacy against biofilms of MRSA and assess the activity of these compounds using an *in vivo* infection model (*Galleria mellonella*).

B146

Investigating *Elizabethkingia spp.* virulence factors

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Abstract

Elizabethkingia spp. are emerging human pathogens, capable of causing meningitis and bacteraemia in neonates and immunocompromised patients with up to 57% mortality. Both *E. meningoseptica* and *E. anophelis* have been implicated in nosocomial outbreaks, having been isolated from both patients and environmental sources such as taps and ventilators. The *Elizabethkingia* genus exhibits multi-drug resistance (MDR), with both extended-spectrum- and metallo-*β*-lactamases conferring innate resistance to penicillins, carbapenems and cephalosporins. Other virulence factors such as biofilm formation, exopolysaccharide synthesis, and efflux pumps grant further resistance to antimicrobials.

Geographically, isolates of both *E. meningoseptica* and *E. anophelis* have different antibiotic resistance profiles, which can delay the administration of efficacious treatment and increase risks of mortality and morbidity. Comparisons of virulence factors between different strains of two species commonly implicated in outbreaks across the world is clinically valuable.

Due to a lack of published breakpoints, zones of inhibitions, minimum inhibitory, and minimum bactericidal concentrations were elucidated to identify resistance and susceptibility of *Elizabethkingia spp.* to a range of antibiotics. Alongside this, biofilm assays were carried out with crystal violet and XTT staining with a view to evaluate any strain or species differences regarding biofilm formation and metabolic activity.

Infection control and prevention are of great importance with the rise of antimicrobial resistance. With the MDR nature of *Elizabethkingia spp.* contributing to its high mortality rates in the immunocompromised, having a clearer understanding of virulence factors may better inform patient care and therefore outcomes.

B147

Escherichia coli pathobiont from dysbiotic intestinal microbiota of severe alcoholic hepatitis exacerbates alcohol gut epithelial barrier damages

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Abstract

Alcohol-associated liver disease (ALD) is increasingly recognized to originate with alcohol-induced intestinal microbiota dysbiosis and epithelial barrier dysfunction called alcohol-associated bowel disease. *Enterobacteriaceae* from patient cohorts was analysed, all excessive alcohol consumers, but some with severe alcoholic hepatitis (sAH) or not (noAH). In a human enterocyte model of the intestinal epithelial barrier, ethanol exposure alone caused moderate structural damage to tight junctions (TJs). However, exposure to an *Enterobacteriaceae* consortium isolated from the stool of a patient with sAH not only intensified ethanol's effects on TJs but also induced cellular damage, particularly the loss of the brush border. Intestinal barrier disruption in this model was determined via immunofluorescence imaging and transwell assays. A diffuse-adherent *Escherichia coli* strain, sAH/DM23, isolated from the sAH consortium, reproduces these lesions. Whole-genome sequencing and phenotypic analyses reveal that sAH/DM23 belongs to the B2 phylogroup, O16:H5 serotype, and multidrug-resistant ST131 clone. Virulence genes were identified including those encoding type 1 pili, F1845 fimbriae (*daa* operon), and serine protease autotransporter toxins Sat and TagC. Mechanistically, serine protease activity disrupts TJs, while calcium-dependent signalling and serine protease activity contribute to brush border loss. These findings identify an *E. coli* pathobiont that exacerbates ethanol-sensitized gut barrier dysfunction, highlighting a potential microbial target for therapeutic intervention.

B148

Targeting Resistant Pathogens Using Peptides and Secondary Metabolites from Ayurvedic Plants.

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Abstract

Antimicrobial resistance remains a significant global health challenge, driving the development of new antimicrobial agents with alternative mechanisms of action. Ayurvedic plants are a rich yet underexplored source of antimicrobial compounds . In this study, we investigated two understudied Ayurvedic Plants, *Aegle marmelos* and *Moringa oleifera*, as potential reservoirs of novel antimicrobial peptides (AMPs).

Genomic data from *A. marmelos (*accession no. ASM3044987v1) and *M. oleifera* (accession no. ASM2139783v1) were retrieved from NCBI, and the open reading frames (ORFs) were predicted using Augustus, followed by Macrel, which was used to predict AMP candidates. These candidates were subsequently filtered for AMPs with a length of ≤ 22 amino acids and were further cross-checked using AMPA and CAMP tools, and assessed for novelty by BLAST, resulting in a total of 81 potential candidates. Nineteen peptides, namely, Aeglicin 1, Aeglicin 4, Aeglicin 6, Aeglicin 7, Tarapcidin α, Tarapcidin σ, and Tarapcidin δ), Moringanin-9, Moringanin-13, Moringanin-14, Moringanin-15, Moringanin-16, Moringanin-17, Moringanin-18, Moringanin-19, Moringanin-21, Moringanin-66, Moringanin-49 and Moringanin-78 demonstrated the most antimicrobial activity with minimum inhibitory concentrations of 125 µg/mL against *Escherichia coli K12, Acinetobacter baumannii, Pseudomonas aeruginosa and* Epidemic Methicillin-Resistant *Staphylococcus aureus (*EMRSA 15).

The findings highlight the antimicrobial potential of plant-derived peptides and support Ayurvedic plants such as *A. marmelos* and *M. oleifera* as promising sources of new agents to combat drug-resistant pathogens.

B149

Evolutionary adaptation of *Listeria monocytogenes* to L-isoleucine starvation reveals a complex regulatory interplay between the SigB and CodY

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Abstract

Listeria monocytogenes is a ubiquitous foodborne pathogen well-known for its remarkable ability to adapt to a wide range of environmental stresses. This stress response is largely controlled by an alternative sigma factor Sigma B (SigB). We screened two laboratory reference strains of *L. monocytogenes*, EGD-e and 10403S, belonging to distinct clonal complexes (CC9 and CC7) and found that both are unable to grow without isoleucine (namely isoleucine pseudo-auxotrophy), even though they possess intact isoleucine biosynthetic pathways (*ilv*). The global regulator CodY (function as a branched-chain amino acid and GTP sensor) have previously been shown to regulate expression of the *ilv* operon. In this study we aim to further clarify the regulatory mechanisms through *in-vitro* evolution experiments (IVEE) and whole-genome sequencing (WGS) of isolated suppressors in the EGD-e background.

We sequenced 10 mutants that displayed shorter-lag in the absence of isoleucine. These included SigB loss-of-function mutations (*rsbT*A42D, *rsbT*Y96*, and *sigB*G156*), and mutations in the manganese-uptake system (*mntA*E255K and *mntA*K286 frameshift), which may influence SigB activity through RsbU. Elevated *ilvD* transcriptional activity in isoleucine-deficient medium was observed in both *mntA* and *sigB* loss-of-function mutants by qPCR, suggesting a possible regulatory interaction between SigB and CodY. To investigate this interplay, we employed egfp-based transcriptional reporter to monitor SigB activity under various growth conditions. Together these data suggest a complex regulatory network involving both CodY and SigB and reveal that short term genetic adaption can overcome the block in isoleucine biosynthesis that is present in wild-type *L. monocytogenes* strains.

B150

Shielded by Design: How Surface Proteins Facilitate Complement Evasion in *S. aureus.*

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Abstract

Staphylococcus aureus causes various infections, and unlike its close relative*, S. epidermidis, S. aureus* produces several complement evasions (CE), including secreted and cell-wall proteins, to inhibit the complement cascade. However, the hierarchy of cell-wall proteins and the role of host extracellular matrix (ECM) proteins in mediating immune evasion remains unclear. Therefore, we aim to (1) study the hierarchy of surface proteins in *S. aureus* that limit complement deposition and (2) understand how ECM proteins affect its evasion of complement.

Using a complement deposition assay combined with flow cytometry and isogenic mutants lacking individual cell wall–anchored (CWA) proteins, we assessed how these surface proteins contribute to resistance against complement activation. This analysis revealed a hierarchy of influence on complement deposition, with Spa (Protein A), Sbi (*Staphylococcal* binder of immunoglobulin), and ClfA (clumping factor A) identified as the most influential proteins. Specific CEs bind to ECM components such as fibrinogen (Fg) and fibronectin (Fn), with several CEs involved in recruiting the host complement regulator, Factor H (FH). The presence of Fg and Fn facilitates immunomodulation at the bacterial surface, highlighting the intricate connection between ECM recruitment and complement deposition. Importantly, we have not identified a dedicated surface protein responsible for FH recruitment; FH binding to the staphylococcal surface occurs only when complement is activated and the bacterial surface is opsonised with C3b/C3d.

These findings enhance our understanding of how cell wall-anchored proteins protect *S. aureus* from complement opsonisation and could guide the development of future immunotherapies.

B151

Regulation of Danger Signal Secretion During Bacterial Infection

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Abstract

When under control, release of ATP results in localised inflammation that acts in a protective manner to instigate the clearance of infection. However, when dysregulation occurs, for example because of mutations in PIEZO1, uncontrolled inflammation becomes damaging and can lead to conditions such as Crohn's disease. Further understanding of these inflammatory mechanisms are required before more effective strategies to manage either scenario can be achieved.

We have recently shown that ATP is secreted by intestinal epithelial cells when infected by invasive gut bacteria as an early immune response. The mechanosensor PIEZO1 can detect the membrane ruffles that occur upon invasion by bacteria such as *Shigella flexneri* and *Listeria monocytogenes*, allowing Ca^{2+} influx and resulting in the rapid release of ATP.

It is currently unknown whether PIEZO1 is activated by enteropathogenic *Escherichia coli* (EPEC), which is non-invasive but still causes structural changes to the cell membrane with the formation of an actin pedestal to which bacteria adhere. EPEC is of interest because, unlike for *S. flexneri*, genetically amenable in vivo infection models are available. Additionally, other innate immune pathways may synergise with PIEZO1. Our goal is to unravel whether and how PIEZO1 and immune sensors activation contribute to ATP secretion and inflammation during EPEC and *S. flexneri* infection.

Understanding these new pathways regulating ATP secretion will provide novel ways to fight infection and possibly inflammatory intestinal diseases.

B152

Characterisation of the bacterial SOS response in clinical isolates of carbapenemase-producing *Klebsiella pneumoniae*

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Abstract

Klebsiella pneumoniae is a major cause of multidrug resistant, hospital-acquired infections which is estimated to cause bacteraemia in 7.1 cases per 100,000 people. Fluoroquinolones, a commonly used class of antibiotics to treat *K. pneumoniae* infections, are potent inducers of the SOS response, which is a stress-induced DNA repair pathway repressed by LexA under normal conditions. Following single-stranded DNA damage, RecA activation triggers self-cleavage of LexA, derepressing *umuDC*, which encodes DNA polymerase V, an error-prone polymerase that enables translesion synthesis resulting in mutations that may increase genetic diversity within populations. Understanding this response in clinical isolates is key to determining how antibiotic pressure drives mutagenesis and evolution of antimicrobial resistance. This study characterised the SOS response in 12 clinical carbapenemase-producing *K. pneumoniae* isolates. Comparative genomic analyses with *Escherichia coli* MG1655 revealed a conserved SOS network, including *lexA, recA* and *umuDC* in all isolates. However, unlike *E. coli*, up to three chromosomal *umuDC* homologues were identified in some isolates, alongside additional plasmid-borne homologues located on plasmids frequently carrying antimicrobial resistance genes. Across all isolates, three to six *umuDC* homologues were detected and preserved UmuDC structural domains were also identified. Conserved LexA-binding motifs matching the *E. coli* consensus sequence were found, which indicated maintained LexA regulation. Ongoing phenotypic assays investigating the effects of *umuDC* copy number on mutagenesis and evolution of antimicrobial resistance in *K. pneumoniae* are ongoing.

B153

Novel insights into RSV infection of macrophages and consequential RSV restriction and epithelial cell cytopathogenesis in a co-culture model.

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Abstract

Background: Respiratory syncytial virus (RSV) is a leading cause of severe lower respiratory tract infections worldwide. Disease severity is associated with excessive and dysregulated immune cell influx into the lungs. This study examined the susceptibility of monocytes and macrophages to RSV infection in vitro and their role in epithelial–immune cell interactions.

Methods and Results: Monocyte-like and macrophage-like THP-1 cells were exposed to RSV A2/mKate2 (MOI 0.1 or 1), which expresses a red fluorescent protein. Monocyte/macrophage infection, virus spread, and infectious virus production were evaluated using Celigo fluorescence imaging and virus titration. Macrophage-like THP-1 cells supported productive RSV infection, in contrast to monocyte-like cells, indicating increased susceptibility after differentiation. To assess their impact on epithelial infection, A549 and BEAS-2B cells were infected and then co-cultured with monocytes/macrophages in direct contact. Imaging suggested RSV transmission from epithelial cells to macrophages. Co-culture with macrophages, but not monocytes, reduced viral spread while causing substantial epithelial damage, including loss of confluency and cell elongation, compared to RSV-infected epithelial monocultures. No epithelial damage occurred in uninfected co-cultures. Macrophages formed dense clumps in infected co-cultures, in contrast to larger, looser aggregates in uninfected cultures.

Conclusions: Macrophages are susceptible to productive RSV infection, which may promote viral spread. However, once infected, they restrict viral propagation while causing severe epithelial cell cytopathogenesis. Future work will characterise monocyte and macrophage activation states, validate these findings using primary airway epithelial cells and PBMC-derived monocytes and macrophages, and explore the mechanisms driving RSV restriction and macrophage-mediated epithelial cell damage.

B155

Motility and invasion of *Streptococcus gallolyticus* subspecies *gallolyticus* in the presence of induced extracellular ATP release from colorectal cancer cells.

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Abstract

Streptococcus gallolyticus subspecies *gallolyticus* (*Sgg*) is an opportunistic gut pathogen associated with colorectal cancer (CRC). Through colonisation of the CRC tumour, *Sgg* disseminates through the bloodstream causing bacteraemia and infective endocarditis. Our lab identified that *Sgg* displays twitching motility via a Type IV Pilus and can invade CRC cells, while the presence of excess glucose and nucleotides inhibits both.

PIEZO1 is a mechanosensitive membrane channel that, when activated, results in extracellular ATP (eATP) release from cells as an early immune response during bacterial invasion. We hypothesised that this may protect colonic epithelial cells against *Sgg* invasion. To test this, HT-29, a CRC cell line, was treated with PIEZO1-agonist Yoda1, or Yoda1-competitive inhibitor, Dooku1, prior to *Sgg* gentamicin protection assays. The supernatants of treated cells were also harvested and used in motility and eATP quantification assays.

Following Yoda1 treatment, HT-29 invasion was significantly reduced compared to control, while an increase was observed after Dooku1 treatment. Importantly, Yoda1 caused a significant increase in eATP released by HT-29 cells, while an increase was also observed during *Sgg* invasion alone. As PIEZO1-agonist, Yoda1, led to increased eATP released from CRC cells, it is likely that this eATP is playing a role in the reduced invasive capacity of *Sgg*. This is the first time DAMP release has been seen to directly inhibit bacterial pathogenesis. The downstream ATP release effector is likely a connexin/pannexin channel present in the membrane which warrants further investigation. This study furthers our understanding of the pathogenesis of *Sgg* in CRC.

B156

Targeting Endodontic Pathogens: The Antimicrobial Potential of Tricalcium Silicate–Based Endodontic Sealers

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Abstract

Aim:
To evaluate the antimicrobial properties of Tricalcium silicate–based endodontic sealers used in root canal treatment.

Methodology:
Two single-syringe sealers (AH Plus Bioceramic, Dentsply; and BioRoot Flow, Septodont) were compared with hand-mixed counterparts (AH Plus and BioRoot RCS). Microstructural and compositional analyses were conducted using scanning electron microscopy (SEM) and energy dispersive spectroscopy, while crystalline phases and chemical changes were characterised via X-ray diffraction and Fourier-transform infrared spectroscopy. The pH was also monitored. Antimicrobial activity was assessed using a direct contact assay against *E. faecalis* and *F. nucleatum* (mono-species set up), and a multispecies biofilm model comprising *E. faecalis*, *P. gingivalis*, *F. nucleatum*, and *S. oralis*. Tests were performed at 0 and 28 days (n = 3). Statistical analyses included ANOVA with Tukey post hoc and Kruskal–Wallis tests.

Results:
All sealers contained zirconium, calcium, and silicon. SEM showed surface porosity and cracking over time. BioRoot RCS exhibited calcium hydroxide peaks and a higher pH ($p < 0.05$) at later intervals. BioRoot RCS demonstrated the strongest antimicrobial effect, particularly against *E. faecalis* and *F. nucleatum*, whereas AH Plus showed the least.

Conclusion:
Calcium silicate–based sealers displayed superior antimicrobial activity compared with conventional epoxy resin–based sealers, highlighting their potential advantage in root canal disinfection.

B157

Bacterial warfare plasmids are associated with virulence and antimicrobial resistance

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Abstract

Bacteria have evolved diverse weapons to kill or inhibit competitors. These weapons are often encoded on large mobile genetic elements that carry additional genes and functions. Yet, the evolutionary dynamics of these elements and their influence on bacterial ecology remain poorly understood. To address this, we investigated 1000s of bacteriocin plasmids in *E. coli* and other Enterobacteriaceae. Although bacteriocins occur broadly across *E. coli* strains, their presence is strongly enriched in pathogenic extra-intestinal (ExPEC) lineages, including many clinically-important pandemic strains. These lineages frequently possess large plasmids that co-encode bacteriocins, virulence factors including siderophores, and antimicrobial resistance genes. Notably, combinations of siderophores and bacteriocins targeting siderophore receptors have evolved independently multiple times and persist in specific lineages, likely conferring a competitive advantage in complex environments such as the inflamed human gut. Across all strains, we detect numerous orphan immunity proteins, underscoring the ecological significance of bacteriocin-mediated antagonism. Furthermore, we provide evidence for frequent horizontal transfer of bacteriocin genes between strains via plasmids and between plasmids via transposons. Several bacteriocins are also shared with *Salmonella enterica*, reinforcing their association with virulence. Collectively, our findings highlight bacteriocins as key determinants of microbial community structure and potent weapons in antimicrobial-resistant pathogens.

B158

Pathogen and parabiotic components differentially prime host immunoreactivity.

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Abstract

Background:
Infection-induced inflammation is essential for host defence but can trigger excessive responses, driving immunopathology through cytokines (e.g., TNF-α, IL-6, and IL-1β). Conversely, non-viable probiotics (parabiotics) and their biofilm may exert immunosuppressive activity. However, the cellular mechanisms underlying differential host recognition of pathogen vs parabiotic components remain unclear.
Methods:
Heat-killed components and biofilms from three pathogens (*Staphylococcus aureus*, *Acinetobacter baumannii*, *Candida albicans*) and four probiotics (Saccharomyces boulardii, *Streptococcus thermophilus*, *Bifidobacterium lactis, and Lactobacillus acidophilus*) were obtained under standardised conditions. Metabolites from biofilm preparations normalised by protein content were analysed via LC–MS. Immunoreactivity against microbial culture supernatants was assessed by Western blotting using sera from healthy donors (HDs; n=16). Flow cytometric analysis of functional biomarkers (MHC-II, CD80, CD206, Dectin-1, CD44, CD25, T-bet, FoxP3, ROR-ɣt, IFN-ɣ and TNF-α) in murine peritoneal cells (PCs) exposed to microbial components is underway.

Results:
Standardised growth kinetics ensured reproducible material generation and CFU experimental normalisation. LC–MS profiling revealed distinct metabolite signatures separating prokaryotic and eukaryotic biofilms, with clear species-specific clustering. Western blotting demonstrated strong HD serum reactivity to pathogen-derived secreted components (*C. albicans* 93.8%, *A. baumannii* 93.8%, S. aureus 87.5%), and minimal reactivity to probiotic counterparts (*B. lactis* 62.5%, none to *S. boulardii, S. thermophilus, L. acidophilus*) ($p < 0.0001$). Preliminary PC analyses support that specific probiotics dampen pathogen-driven responses.

Conclusion:
Host immune recognition distinguishes pathogen-associated from parabiotic-derived components. The low antibody reactivity to parabiotics supports their potential as immunomodulatory agents. Ongoing analyses will further define how probiotic components suppress pathogen responses.

B159

Lineage-specific sensitivity towards Acetate and Lactate in virulent and resistant *Listeria monocytogenes*: A comparative phenotypic-genotypic analysis.

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Abstract

Background*: Listeria monocytogenes* is a Gram-positive, rod-shaped, intracellular, foodborne pathogen with robust stress response mechanisms. It is important to study acid resistance since weak acids play an important role in food preservation, along with encountering weak acids in hosts' innate barrier in the stomach.

Methods: A collection of 224 *L. monocytogenes* strains collected over the years by our lab together with collaborators, will be used in this study. Phenotypic characteristics were tested by an agar-based assay to investigate their growth & survival properties in presence of a range of organic acid food preservatives and food sanitizers. High-quality images of all the plates were scanned every 24 h and pixel intensity were measured using ImageJ.

Results: Range of sensitivity against acetic and lactic acid was observed. Lineage II strains (n=102) had significantly poorer growth after 72 h on BHI agar with 30mM acetic acid at pH 5.8 compared to Lineage I strains (n= 94; $p < 0.05$). This difference was even larger when only clinical isolates were compared between the two lineages. Lineage I clinical strains were more resistant to acetic acid than lineage II strains ($p < 0.01$). In contrast, Lineage II strains were significantly more resistant to lactic acid (25mM at pH 5.8) than Lineage I strains ($p < 0.01$) based on colony growth recorded at 72h.

Conclusions: Wide range of sensitivity was detected in the whole strain collection. Genome sequence analysis between closely related strains that differ phenotypically with respect to acetate, lactate, peroxyacetate resistance are underway and would be presented.

B160

Mode of Action of Lymphostatin: a Novel Lymphocyte Inhibitory Factor of Pathogenic *Escherichia coli*

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Abstract

Attaching and effacing (A/E) *Escherichia coli* are a frequent cause of diarrhoeal disease worldwide. Lymphostatin (LifA) is a large (365 kDa) multi-domain virulence factor found in most enteropathogenic (EPEC) and many non-O157 enterohaemorrhagic (EHEC) *E. coli* strains. LifA inhibits mitogen- and antigen-stimulated activation of bovine and human T lymphocytes *in vitro*. In addition, LifA and LifA-like, a homologue also found in EPEC, play an accessory role in A/E lesion formation on human intestinal mucosa. LifA contains an N-terminal glycosyltransferase domain hypothesised to glycosylate unknown host signalling molecules, and a cysteine protease domain which facilitates autocatalytic cleavage and cytosolic release of the N-terminal fragment following endocytosis. A C-terminal ADP-ribosyltransferase (ART) domain was also recently identified but remains functionally uncharacterised. This project aims to address key gaps in understanding of the mode of action of LifA, and to further characterise LifA-like. Using AlphaFold 3 structural predictions, we identified conserved glycosyltransferase and cysteine protease domains in LifA homologues LifA-like, ToxB (EHEC O157:H6) and CPE2_0552 (*Chlamydia pecorum* W73). We cloned, expressed, and purified recombinant LifA-like, and will assess its inhibitory activity against bovine T lymphocytes *in vitro*. Using Cy3B-maleimide-labelled LifA, we demonstrated for the first time that LifA uptake by bovine T lymphocytes occurs in a manner consistent with receptor-mediated endocytosis. Finally, we cloned, expressed, and purified a LifA-ΔART mutant protein to investigate the potential role of the ART domain in T lymphocyte inhibition and in A/E lesion formation on intestinal epithelial cells.

B161

Immunosuppression persists despite recovery in patient with Staphylococcus aureus bacteremia sepsis.

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Abstract

Background: Sepsis is an infectious condition affecting147,000 individuals in the UK, ~40,000 of whom would die. During sepsis, proinflammatory cytokines (e.g., IL-1 and TNFα) increase systemically, generating the so-called "cytokine storm". Concurrently, the compensatory anti-inflammatory response (CARS) triggers the expression of inhibitory cytokines (e.g.; IL-10) and cell surface proteins, including checkpoint receptors and their ligands, which is thought to counteract excessive immune responses. Yet, the kinetics and significance of the expression of multiple inhibitory pathways in sepsis patients remains unclear.

Method: We analysed immune responses of Staphylococcus aureus bacteremia sepsis patients (N=8), during the first two weeks of hospital stay, in comparison to healthy donors (HDs, N=10). We designed multiparametric flow cytometry panels to study kinetic variations of the phenotype of monocytes/macrophages and T cells isolated from the blood of sepsis patients, focusing on expression of multiple checkpoint receptor expression.

Results: Although CD14+ CD16+ monocytes/macrophages numbers increased during sepsis, these expressed less markers of activation (HLA/DR, CD40, CD86) and more checkpoint proteins than healthy donors. T cells also expressed higher amounts of multiple checkpoint receptors in the first week of sepsis. Consistent with dominant inhibitory pathways, SA-specific responses remained low and hard to detect in patients, while SA-toxin antigens converted CD4+ T-cell populations into suppressive regulatory T-cells (Tregs) in sepsis patients more so than in HDs.

Conclusion: Our study suggests that immune cells from sepsis patients maintained a predominantly immunosuppressive phenotype during hospital stay, which persisted despite recovery.

B162

Aspergillus fumigatus enhances non-typeable *Haemophilus influenzae* growth during interkingdom biofilm formation

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Abstract

Non-typeable *Haemophilus influenzae* (NTHi) and *Aspergillus fumigatus* are key contributors towards chronic lung diseases such as COPD and CF, with their developing resistance towards antimicrobials of growing global concern. Recent evidence has shown that NTHi and *A. fumigatus* frequently co-exist in fungal balls isolated from chronic rhinosinusitis patients, suggesting that the formation of interkingdom biofilms may be of clinical importance. Interactions between *A. fumigatus* and other bacterial species including *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Klebsiella pneumoniae* have previously been investigated, however, its interaction with NTHi remains unexplored. To investigate this, we established an interkingdom biofilm model to examine the interplay between *A. fumigatus* and NTHi, hypothesising that their co-culture would provide a synergistic environment. Interkingdom biofilms were grown in chemically defined media for 24 and 48 hours, seeding with either *A. fumigatus* conidia or hyphae. Viability assays indicated that the NTHi population increased when grown in co-culture with *A. fumigatus* conidia, however, confocal microscopy revealed the absence of either conidia or hyphae, suggesting that NTHi displays predatory behaviour in breaking down conidia to support its growth. In contrast, seeding with *A. fumigatus* hyphae increased NTHi viability and metabolic activity (measured through XTT), with confocal imaging revealing a dense network of hyphae interspersed with large populations of NTHi, confirming their capacity for developing mixed species biofilms. The development of this interkingdom model has provided new insight into the interactions between *A. fumigatus* and NTHi, serving as a basis for future studies investigating their role in infection and the assessment of treatments.

B163

Surveillance of Influenza A in Irish Wildlife: Insights from the OH-ALLIES initiative.

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Abstract

Highly pathogenic avian influenza (HPAI) represents an ongoing human and animal health concern globally. Although primarily affecting avian hosts, increasing detections in wild and domestic mammals suggests expanding host range and interspecies transmission dynamics. The One Health All Ireland for European Surveillance (OH-ALLIES) initiative conducts systematic surveillance of wild mammals and birds in Ireland to characterise influenza A virus (IAV) exposure and infection dynamics, assess risk and inform biosecurity and public health strategies.

Between August 2024 and March 2025, tissue, serum and swabs samples were collected from over 1000 animals, primarily red foxes, American mink, Eurasian badger and feral cats. Exposure and active infection were assessed using serological and molecular methods. Serology included a commercial IAV ELISA, with subtype-specific testing for H5, H7 and H9. Real-time PCR targeting the IAV matrix gene was used to detect viral RNA. To address field sampling challenges, Nobuto filter papers were evaluated as an alternative to serum for antibody detection.

To date, 11 of the 1073 animals (10 foxes, 1 badger) were seropositive for IAV antibodies, with H5 antibodies confirmed in seven. Most seropositive foxes (n=9) were found within 1km of the coast. Nobuto filter paper testing showed 98.2% agreement with paired serum samples, demonstrating its strong potential for wildlife surveillance. No viral RNA has yet been detected by RT-PCR.

These findings highlight the potential for IAV exposure among wild mammals in Ireland, and demonstrate the importance of integrated surveillance approaches to assess spillover potential and inform ongoing One Health monitoring.

B164

A 3D printed co-culture model of cystic fibrosis lung infection: analysis of *Pseudomonas aeruginosa* and *Staphylococcus aureus* interactions.

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Abstract

The complex interkingdom systems that enable biofilm persistence within the lungs of people with cystic fibrosis remain largely unknown. What is known is that the virulence of key pathogens, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus,* is driven by the unique biofilm structure they form. Chronic colonisation by these species from childhood is sustained through the formation of multispecies biofilms, which promote genetic variation in the highly mutable *P. aeruginosa*, enhancing the virulence of both *P. aeruginosa* and *S. aureus* as well. To understand the interaction between the two species when in direct and indirect contact, we have established a 3D-printed co-culture of both species. Spatial distribution of the microbes is achieved by utilising a digital light processing (DLP) 3D printer to layer microbes in a photocurable monomer. We have used this model to understand the difference in gene expression of both *P. aeruginosa* and *S. aureus* when in direct and indirect contact through RNA sequencing. The results indicate that *S. aureus* has many differentially expressed genes when in indirect and direct contact with *P. aeruginosa* for 24 hrs, whereas *P. aeruginosa* only has a significantly different gene expression profile when in direct contact with *S. aureus*, suggesting a response to diffusible substances from *P. aeruginosa*. Differentially regulated gene groups suggest a change in global metabolic status due to direct interactions between the species. Further work will utilise mass spectroscopy to understand the effect of indirect and direct contact on quorum-sensing molecules and biofilm formation within a 3D printed structure.

B165

Pre-symptomatic detection of urinary tract infection post-kidney transplant using qPCR

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Abstract

Urinary tract infections (UTIs) remain one of the most common and clinically significant complications following kidney transplantation, impacting between 25-75% of recipients. In turn, this increases risk of acute organ rejection and associated morbidity, raises healthcare costs, and contributes to the antimicrobial resistance (AMR) crisis. The problem is further exacerbated by the use of essential urological devices, such as stents and catheters, which provide adherent surfaces for infection-causing bacteria.

Typically, UTIs are not diagnosed until a patient becomes symptomatic, primarily using culture-based approaches. This approach is time-consuming as several days are required for the bacteria to grow, followed by identification and antimicrobial susceptibility testing. These delays can postpone targeted antibiotic therapy, allowing infections to progress in immunocompromised kidney transplant (KT) recipients. Further, traditional diagnostic thresholds, such as 10^5 CFU/ml, may not capture early-stage infections. However, genetic approaches such as quantitative PCR (qPCR) are becoming widely adopted in clinical practice due to their enhanced sensitivity and speed when compared to traditional approaches.

Therefore, we employed a multiplex qPCR approach, combining and validating existing assays targeting 16S rRNA, *Escherichia coli*, and *Klebsiella pneumoniae*, two uropathogens accounting for over 50% of UTIs in KT recipients. By detecting pathogens below the clinical threshold for diagnosis, this approach allows for earlier and more precise antimicrobial intervention, reducing the need for broad-spectrum antibiotics. Ultimately, this work demonstrates how multiplex genetic approaches can enhance early diagnosis, guide treatment options, and improve post-transplant care.

B166

Standardised Screening of Antimicrobial Peptides Against *Candidozyma auris* and *Nakaseomyces glabrata*

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Abstract

Candida infections impose a substantial burden (~750,000 cases/year; invasive candidemia ~42% mortality).

Nakaseomyces glabrata (formerly *Candida glabrata*) and *Candidozyma auris* (formerly *Candida auris*) are World Health Organization high-priority pathogens for their emerging drug resistance and increased incidence.

Antimicrobial peptides (AMPs) are short, cationic, and amphipathic peptides that exert multimodal mechanisms distinct from commonly used antifungals.

We collated 964 unique anti-*Candida* peptide sequences were exported from the Antimicrobial Peptide Database (APD3). Restriction criteria (sequence length between 10 to 25 amino acids in length, MIC in *C. albicans* of 16 µg/mL or less, minimal toxicity within 8-fold MIC, and exclusion of cysteine rich or heavily modified sequences) yielded 77 peptide candidates for anti-*C. auris* and *N. glabrata* activity assessment.

Using the CLSI broth microdilution method, we screened 67 of these AMPs against *C. auris* strains (DSM 105986; DSM 21092), *N. glabrata* strains (DSM 24506; DSM 70614), and *C. albicans* (DSM 11946) as a technical control. 45 of the 67 peptides were active at ≤256 µg/mL against at least one species: 13 were active against *C. auris* and 27 against *N. glabrata*. All peptides were active against *C. albicans*. 3 AMPs were found to be potent against all three at concentrations between 16-128 µg/mL

By standardising our method, we generated directly comparable MICs and a robust, rank-ordered dataset to select the most potent candidates for rational design and optimisation as MDR Candida therapeutics.

B167

Detection and characterisation of Non-O157 Shiga Toxin-Producing Escherichia coli on Scottish Deer Farms

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Abstract

Shiga-toxin producing *Escherichia coli* (STEC) are a group of zoonotic enteric pathogens that cause foodborne human infections globally. In the UK, STEC serogroup O157 (STEC O157) strains are responsible for most clinical cases, however the incidence of non-O157 STEC clinical cases has increased in recent years.

Ruminant livestock are considered the primary reservoir of STEC, and deer are now considered an important species in the STEC transmission cycle. We previously estimated the prevalence of STEC O157 and non-O157 STEC in wild Scottish deer as 0.28% and 69.5%, respectively. In contrast, STEC O157 was detected in 9.65% of Scottish farmed deer, closer to the levels present in Scottish cattle.

We aimed to detect and characterize non-O157 STEC strains present on Scottish deer farms. Faecal samples were collected on 10 Scottish deer farms, four of which were mixed enterprises that also farmed cattle and/or sheep. Samples were enriched, plated on SMAC agar and colonies screened for the presence of *stx1*, *stx2* and *eae* genes. Whole genome sequencing was performed on two positive isolates per species from each farm. Additional isolates were also collected from mixed enterprises to investigate the impact of cattle and/or sheep on non-O157 STEC diversity.

To date, >80 non-O157 STEC strains have been identified across 10 deer farms. Initial sequencing of 30 strains has identified 16 different O-types. Further sequencing is underway to determine whether strains are host-species specific, and to evaluating public health risk by assessing genetic relatedness to strains associated with human clinical disease.

B168

Screening for Antibiotic Resistance Genes and Mechanisms in Gram-negative Bacteria Isolated from Ugandan Neonatal Sepsis.

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Abstract

Gram-negative bacteria (GNB) such as *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter* spp. are leading causes of neonatal sepsis in low-to-middle-income countries (LMICs). Increasing antimicrobial resistance (AMR) among these species poses a growing public health burden due to their ability to acquire and transfer resistance genes through horizontal gene transfer. Resistance to first-line neonatal therapeutics is mainly associated with extended-spectrum β-lactamases (ESBLs) and aminoglycoside-resistance genes, while carbapenemases threaten last-resort antibiotics used for multidrug-resistant infections.

Blood samples were collected from neonates with suspected sepsis in Uganda between 2019 and 2021 and identified by microscopy and MALDI-ToF. Minimum inhibitory concentrations (MICs) were determined for a defined antibiotic panel. Meropenem-resistant isolates were screened by multiplex PCR for $bla_{\text{NDM-1}}$, bla_{KPC} and $bla_{\text{OXA-48-like}}$, β-lactam-resistant isolates for $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$ and $bla_{\text{SHV-1}}$, and aminoglycoside-resistant isolates for *aac(6')-Ib*, *aph(3')-Ia* and *armA*. Whole-genome sequencing (WGS) was performed on selected isolates.

Of the GNB, 98.7% were β-lactam resistant, mediated by $bla_{\text{CTX-M-15}}$ (61/225), $bla_{\text{TEM-1}}$ (114/225) and $bla_{\text{SHV-1}}$ (28/225). Aminoglycoside resistance occurred in 76/236 isolates, with *aac(6')-Ib* (21/76), *aph(3')-Ia* (10/76) and *armA* (15/76) detected. Eleven isolates were meropenem resistant; none carried $bla_{\text{OXA-48-like}}$ or bla_{KPC}, while five *Acinetobacter* spp. harboured $bla_{NDM\text{-}1}$.

The high prevalence of ESBL-producing GNB highlights the urgent need to revise empirical neonatal treatment guidelines in Uganda. β-lactams are unlikely to be effective, whereas gentamicin remains a reliable first-line therapy. Carbapenems should be reserved for confirmed gentamicin-resistant infections, with continued resistance monitoring and strengthened antimicrobial stewardship to safeguard treatment efficacy.

B169

Simulating host environment and its impact on Pseudomonas aeruginosa biofilms

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Abstract

Pseudomonas aeruginosa causes chronic infections in immunocompromised patients, including in the lungs of people with cystic fibrosis, in non-healing wounds, and on endotracheal tubes in intubated patients. Studies have investigated *P. aeruginosa* biofilm using standard laboratory media, thereby neglecting the effect of the environments encountered *in vivo*. In this study, we explored biofilm formation, virulence factor, gene expression, and antibiotic susceptibility of *P. aeruginosa* across three host-mimicking media (HMM).

These were synthetic wound fluid, synthetic cystic fibrosis medium (SCFM), and synthetic ventilated airway mucus (SVAM), which mimic wound fluid, lung secretion of people with cystic fibrosis, and airway liquid of intubated patients, respectively. There were marked differences in the biofilm structure and composition (polysaccharides, eDNA, and proteins) of *P. aeruginosa* grown in different HMM, and there were also differences between strains. Siderophore production was highest in SCFM, while protease production was upregulated in SVAM. Antibiotic susceptibility testing revealed a 2-to 8-fold increase in tolerance to colistin and tobramycin in HMM compared to standard laboratory medium.

We combined the SVAM with a section of endotracheal tube (ETT) to explore clinical strains from patients with ventilator-associated pneumonia (VAP). Biofilms formed on ETT model showed high tobramycin tolerance (512µg/ml), but pretreatment with glycoside hydrolases enhanced susceptibility (64µg/ml), consistent with a key role for exopolysaccharides in biofilm antibiotic tolerance in this environment.

Our study revealed specific ways *P. aeruginosa* modulates its biofilm at different infection sites and proposed the use of relevant biofilm model in routine AST and drug development pipeline.

B170

Investigating biocide mechanism of action in antimicrobial surfaces on bacterial pathogens

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Abstract

Chlorhexidine (CHX) is a widely used biocide with a broad spectrum of activity against bacteria and fungi, as well as some viruses and protozoa. It is commonly used in clinical settings for disinfection and topical antisepsis. Recent advancements have enabled preparation of antimicrobial surfaces containing CHX as the active component. These exhibit strong biocidal activity against a range of microorganisms, including bacterial strains normally resistant to CHX in solution. The mechanism of action of CHX in this context is unclear, as well as how this may vary against different species.

To address this, several bacterial pathogens of clinical relevance were continually exposed to sub-inhibitory concentrations of CHX to promote adaptation, followed by whole-genome sequencing to identify mutations responsible for resistance. These CHX-adapted strains were then assayed against surfaces functionalised with CHX to identify complementary resistance mechanisms.

Intriguingly, susceptibility to CHX in solution did not appear to correlate with sensitivity to CHX surfaces. Additionally, whilst CHX adaptation was observed in multiple independent replicates of several organisms, only one isolate appeared to acquire cross-resistance to surface-bound CHX. These results suggest that CHX has two distinct mechanisms of action when utilised in solution or on antimicrobial surfaces. Analysis of the observed mutations provides novel insight into the biological structures associated with susceptibility and resistance to CHX in both contexts, allowing the mechanism of action to be further elucidated.

B171

Bridging the Clinical-Environmental Interface in Urinary Tract Infections: Multi-Drug Resistance Patterns and MARI Profiling among Diabetic and Non-Diabetic Patients in a Tertiary-Care Hospital of Gujarat, India

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Abstract

Antimicrobial resistance (AMR) remains a critical global threat, particularly within urinary tract infections (UTIs) where empirical antibiotic use is common and diabetic patients are disproportionately affected. This cross-sectional study investigated the prevalence of uropathogens, multi-drug resistance (MDR) patterns, and Multi-Antibiotic Resistance Index (MARI) values among diabetic and non-diabetic UTI patients in a tertiary-care hospital in Gujarat, India. Uropathogens were identified using standard microbiological methods and antibiotic susceptibility testing, while environmental samples were collected from hospital low-touch and high-touch surfaces to assess their contribution to resistant organism dissemination. Among all processed UTI samples, *Escherichia coli* predominated (78%), followed by *Enterococcus* spp. (9%), *Klebsiella* spp. (8%), other Gram-negative organisms (3%), *Pseudomonas aeruginosa* (1%), and *Candida* spp. (1%). Diabetic patients demonstrated a higher incidence of MDR isolates and significantly elevated MARI values compared with non-diabetic individuals, indicating greater antibiotic pressure and increased exposure to resistant reservoirs. Environmental surveillance showed that high-touch areas harboured a substantially greater load of AMR organisms than low-touch sites, implicating these surfaces as potential environmental reservoirs facilitating cross-transmission. The integration of patient-level microbiological findings with environmental resistance data highlights the complex interplay between host vulnerability, pathogen ecology, and environmental persistence within healthcare settings. These findings underscore the need for strengthened infection-prevention practices, routine environmental monitoring, and targeted antimicrobial stewardship strategies, particularly for high-risk populations such as diabetics. Implementing these measures could significantly mitigate AMR burden, reduce infection recurrence, and support evidence-based interventions in resource-limited hospital environments.

B172

Incidence, Risk Factor and Antibiotic Sensitivity trends among Surgical Site Infections in a tertiary care hospital in India

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Abstract

Surgical Site Infections (SSI) are a major concern of healthcare providers due to its huge impact on morbidity, mortality and additional healthcare costs. SSI generally occur within 30 days post non-implant related surgeries. Despite advancements in surgical techniques and infection control measures, surveillance of SSIs remains of paramount importance to analyze and understand the changing patterns of microbial infections and their antibiotic resistance. The present study aimed at estimating the incidence of SSI in a post-operative complication in various surgery departments of a tertiary teaching hospital in India. Out of 2257 surgeries performed in a span of 3 months, 160 pus samples were obtained suspected for SSI for microbiological analysis. Out of the 152 samples, 99 were culture positive. Age, gender, body mass index (BMI), comorbidities, surgical risk as per ASA grading, duration of surgery, wound class and duration of hospital stay in days were recorded for all the samples. The incidence of SSI in our setup was found to be 4.3%. Prolonged surgery (> 2 hours), patient age and comorbid conditions like obesity were found to be significantly associated with SSI, and the clean surgeries showed minimum risk of infection. *Staphylococcus aureus* was the commonest pathogen, followed by *Escherichia coli, Klebsiella spp,* and *Pseudomonas aeruginosa.*Furthermore, antibiotic sensitivity patterns of Gram positive and Gram negative isolates over a spectrum of 38 different antibiotics revealed very distinct patterns. Our findings highlight the increasing problem of antibiotic resistance and promote antibiotic stewardship programmes as essential components for surveillance in healthcare settings.

B173

Automated high-throughput method for assessing pathogenic infectious dose (TCID50) using Celigo imaging cytometer

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Abstract

Qualitative assessment of pathogenic infections, as well as the protective effects of candidate therapeutics, often relies on the observation of specific pathological changes within the host cell. These phenotypic changes, such as cell rounding, swelling/shrinking, granularity are known as a Cytopathic Effect (CPE) and can be visualized via light microscopy. As the magnitude and localization of the CPE may vary considerably, careful examination of replicate samples at various titers is required for reliable, qualitative results. This subjective approach is specific to the infectious agent as well as the host cell is tedious, time consuming, low throughput and requires manual well-by-well examination by highly skilled personnel. In this study, the Celigo imaging cytometer has been used to rapidly assessment of viral infectivity in a range of plate formats. The infectivity level was assessed by characterizing the cell count/ cell morphology and the viral infectivity of Lentivirus concentration was assessed by using a GFP fluorescence reporter to measure % GFP expression in the cells population. Finally, as alternative to TCID50 assays, the viral plaque assay is used to evaluate the number of virus particles by the number of plaques they form. The Celigo imaging cytometer generates uniform whole well brightfield and fluorescent images. By directly counting cells and plaques in whole wells on microplates, the viral infectivity can be quickly quantified avoiding manual subjective counting and image storage can reduce assay time, improve research efficiency and reduce the need to create plaque plates walls in the lab .

Session : Microbial pangenomics: reaching new frontiers of an open and evolving discipline

B174

A genomics-based investigation of acetic acid bacteria across a global fermented food metagenomics dataset

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Abstract

Developing a better understanding of the genomic and metabolic features of acetic acid bacteria (AABs) has the potential to facilitate an improvement of the taste or flavor of fermented foods. Here, we conducted a high-resolution analysis of AABs present in fermented foods based on the investigation of 337 high-quality metagenomic-assembled genomes (MAGs) recovered from 223 metagenomic samples. Firstly, by integrating these MAGs, we built a phylogenetic tree of high-quality MAGs using GTDB-Tk. We found that AABs MAGs from food-related samples and those from other environments are generally phylogenetically distinct, with the majority of those from fermented foods being assigned to a relatively small number of genera. Functional metagenomic analysis also revealed that the fermented food-associated AABs MAGs are associated with the production of carbohydrate-active enzymes, antibiotic resistance genes, and secondary metabolites. Through these investigations, we have gained substantial insights into the diversity, function, and roles of AABs in fermented food microbiomes.

B175

Detecting epistasis across bacterial pangenomes using Continuous-Time Markov Chain Models

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Abstract

Nucleotide covariation can reveal epistasis, exposing how loci combine to generate complex phenotypes such as antimicrobial resistance (AMR). Genome-wide epistasis studies (GWES) in pangenomes typically use mutual information (MI) over k-mers, but MI is sensitive to clonal structure and uneven sampling which can inflate co-occurrence signals or obscure true interactions. We present a phylogeny-aware GWES framework with a continuous-time Markov chain (CTMC) model defined on the inferred tree. By evaluating k-mer pairs via the likelihood of their joint substitution histories, the CTMC approach explicitly accounts for shared ancestry with reduced sampling and population structure biases. Applied to bacterial pangenomes of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli*, the method yields more accurate covariation maps, recovers lineage-specific co-adaptation and detects mutual exclusivity epistasis which was missed by MI. This phylogeny-grounded framework produces function maps of the bacterial pangenome and offers a potential approach to connecting genomic interactions with critical phenotypes such as AMR, complementing experimental microbiology.

B176

Globally disseminated multi-drug-resistant lineages of *Campylobacter*

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Abstract

Campylobacter is the leading cause of bacterial gastroenteritis worldwide, responsible for an estimated 166 million cases annually. *Campylobacter jejuni* and *C. coli* infections are commonly acquired through undercooked poultry, contaminated food, and environmental exposure. Although previous genomic studies have revealed extensive diversity, knowledge of *Campylobacter* population structure and pangenome variation at a global scale remains limited.

Here, we analysed 88,181 *Campylobacter* genomes from 54 countries and eight sources to investigate lineage structure, pangenome composition, and antimicrobial resistance (AMR) gene dynamics. Comparative pangenome analysis revealed that while major clonal complexes are globally distributed, accessory gene content shows strong regional stratification. Ninety-six percent of distinct sequence types (STs) were confined to a single continent, driven largely by differential acquisition of mobile genetic elements and AMR determinants. Globally disseminated livestock-associated lineages such as ST-21, ST-353, and ST-828 exhibit expanding accessory genomes enriched for multidrug resistance and virulence-associated loci, with resistance frequencies increasing sharply in recent years. Across sources, 40–80% of poultry, ruminant, and swine isolates were multidrug-resistant, rising to 70–80% among isolates from South America and Asia.

Together, these results show that the *Campylobacter* pangenome is globally connected but regionally adaptive, with AMR and host-associated genes driving diversification within otherwise cosmopolitan lineages. Understanding these global and regional pan genomic trends will be critical for developing effective interventions to mitigate *Campylobacter* transmission and resistance evolution.

B177

An e-DNA Probe to Enable Microbial Source Apportionment from Environmental Samples

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Abstract

An e-DNA Probe to Enable Microbial Source Apportionment from Environmental Samples

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Abstract

Microbial contamination of waterways threatens public health, agriculture, and environmental integrity. Pathogenic and indicator bacteria such as *Escherichia coli* and *Klebsiella* spp. enter river systems through wastewater discharges and agricultural runoff. Identifying the host origins of these microorganisms remains challenging, limiting effective remediation strategies. This multidisciplinary project aims to develop a rapid, culture-independent method for microbial source tracking (MST) in waterways by integrating advanced genomics with biosensor technology. Two complementary objectives guide the research: firstly, identifying and validating host-specific genetic markers—including accessory genome elements and plasmid-borne genes—that distinguish contamination from human, cattle, pig, sheep, and chicken sources; secondly, developing an electrochemical biosensor for rapid detection of these genetic signatures from environmental DNA in field samples. The methodology employs comprehensive comparative genomic and pangenome analysis of large-scale datasets, focusing on *Klebsiella* spp. due to their ubiquity, diverse mobilome, and significance as opportunistic pathogens. An engineered electrochemical sensor platform will enable multiplexed detection with enhanced sensitivity. Field validation in river catchments with known contamination sources will account for environmental factors affecting bacterial survival and eDNA persistence. This approach aims to enable rapid, on-site identification of contamination sources, facilitating targeted interventions to protect water quality.

B178

Duplication of *bla*I/*bla*R/*bla*Z genes in Methicillin-resistant *Staphylococcus aureus* from humans and animals

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Abstract

BACKGROUND

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains an important pathogen. Gene duplications or multiple gene copies have been shown to contribute to higher antimicrobial resistance. We examined the duplications of *bla*I/*bla*R/*bla*Z and antimicrobial resistance genes (AMR) in MRSA from both humans and animals.

METHODS

Whole genome sequencing (WGS) was performed on 1327 MRSA strains from a One Health project using a combination of Illumina and nanopore sequencing. Of these, 1180 were from bloodstream infections from Hong Kong, 67 were carriage strains from pig farms from mainland China, and 80 were from bloodstream, carriage of humans and pet animals from Sri Lanka. Multi-locus sequence types (MLST) were inferred from WGS. *SCCmec* genes, plasmids and mobile genetic elements were characterized.

RESULTS

The presence of *mec*A gene was confirmed in 1310 MRSA strains. No mecC gene was identified. Ten isolates (0.77%) including nine from humans and one from a pig were identified to carry duplicated *bla*I/*bla*R/*bla*Z genes. These included 9 human strains from Hong Kong and Sri Lanka (inc. five ST772, two ST22, one ST630 and 1 pig strain (ST398) from mainland China. These strains revealed MICs ranging 8 to 512 *u*g/ml to cefoxitin. Gene duplications occurred in tandem and at acquisition events with horizontal gene transfer with mobile genetic elements and prophages. Duplication of other AMR genes was found.

CONCLUSIONS

Gene duplication of *bla*I/*bla*R/*bla*Z genes appears to be a rare event. Duplication of AMR regulatory/resistance genes may play a role in enhanced antimicrobial resistance development.

Session : Microbiome Forum

B180

Vaccine-induced alterations in the fecal microbiota of chickens under commercial rearing conditions

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Abstract

Young chickens raised in poultry farms undergo extensive vaccination programs from early life to maturity to maintain their health. Previous studies have shown that vaccines can alter the gut microbiota of chickens. However, the influence of different vaccines on the gut microbial communities under commercial rearing conditions remains poorly understood. This longitudinal study investigated changes in the fecal microbiota of Hy-Line chickens in response to vaccinations administered under commercial conditions: Infectious Bronchitis/Newcastle Disease/Gumboro disease (IB/ND/IBD) at week 4, Avian Encephalomyelitis/Fowl Pox (AE/FP) at week 7, Chicken Infectious Anemia (CIA) at week 9, and Infectious Bronchitis/Newcastle Disease (IB/ND) at week 12. During each vaccination week, three pooled fecal samples per pen (n=4) were collected from three randomly selected birds both before and after vaccination, and 16S rRNA sequencing was performed. At the phylum level, Bacillota and Fusobacteriota were dominant, while at the genus level, *Lactobacillus*, *Romboutsia*, and *Turicibacter* were highly abundant across samples. Notably, vaccination significantly influenced fecal microbial diversity, with clear differences observed between pre- and post-vaccination ($P<0.05$). Differential abundance analysis further revealed alterations in taxa in the AE/FP, CIA, and IB/ND vaccine groups, whereas no significant changes were observed in the IB/ND/IBD group.

B181

2Pipe: It Starts with a Question. Matching You with the Correct Pipeline for MAG Reconstruction

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Abstract

Whole Genome Sequencing (WGS) has boosted our ability to explore microbial diversity by enabling the recovery of Metagenome-Assembled Genomes (MAGs) directly from environmental DNA. As a result, the vast availability of sequencing data has prompted the development of numerous bioinformatics pipelines for MAG reconstruction, along with challenges to identify the most suitable pipeline to perform the analysis according to the user needs. Here, we present 2Pipe (https://2pipe.app/), an application that provides a compacted technical overview of 44 publicly available pipelines or platforms to build MAGs starting from short and/or long sequences. Moreover, giving the overwhelming number of factors to consider when selecting an appropriate pipeline, 2Pipes includes an interactive decision-support questionnaire that helps users to identify a suitable workflow based on their input data characteristics, desired outcomes and computational constraints. The tool presents as well an interface to customize the recommendation, a pipeline gallery to offer a summarized description, and a pipeline comparison based on key factors used for the questionnaire. Beyond this and foreseeing the release of novel pipelines in the near future, we include a quick form and detailed instructions for developers to append their workflow in the application. Finally, this application equip the researchers with a general outlook of the growing metagenomics pipeline landscape and guide the users towards deciding the workflow that best fits their expectations and infrastructure.

B182

What shapes fish skin microbiomes? Insights from cultured and sequenced three-spined stickleback fish skin microbiome across Scottish lochs.

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Abstract

The fish skin microbiome serves as a protective barrier, influencing host health and facilitating interactions between the host and its environment. While there have been studies on the roles and composition of the fish skin microbiome, there remains a paucity of data on how environmental variation influences these microbes in natural populations. Here, we used culture-dependent and -independent approaches to characterise the skin microbiome of wild three-spined stickleback populations and examine how environmental factors influence microbial diversity and community composition across 17 freshwater lochs on the island of North Uist, Scotland. Culture-dependent methods involve plating a skin microbiome sample on an array of agar followed by sequencing the 16S rRNA gene, whereas culture-independent methods involve sequencing the 16S rRNA gene directly from skin swabs of individual stickleback fish. Analysis of 300 swabs and 450 cultured samples showed that microbial community composition was primarily driven by environmental variables, especially host habitat, water pH, salinity and metal concentrations, while host traits (e.g., sex, length) contributed minimally. A set of dominant bacterial genera—*Janthinobacterium*, *Pseudomonas*, *Acinetobacter*, and *Psychrobacter*—constituted a core skin microbiota across lochs, suggesting that these taxa may play important roles in this microbial community. Culture-based sequencing recovered an average of 87.4% of the amplicon sequence variants (ASVs), representing 99.7% of the relative abundance detected by direct swab sequencing, and additionally identified 51.9% more ASVs not detected in the swab samples. Future investigations of our cultured isolates will explore the functional traits and interactions among these dominant taxa.

B183

Skin Microbiome Dynamics and Strain-Level Dysbiosis in Acne Vulgaris: Evidence from Diverse Egyptian Populations

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Abstract

Background: The role of the skin microbiome, particularly the diversity and strain variability of *Cutibacterium acnes*, has been emphasized in recent research on acne vulgaris pathogenesis. Despite the Middle East and North Africa's unique genetic and environmental traits, acne-related skin microbiome data from this region are lacking.

Objectives: To characterize the facial skin microbiome of Egyptian acne patients and healthy controls, identify microbial signatures linked to acne severity, and assess urban versus rural differences.

Methods: Cheek swabs from 45 acne patients and 25 healthy controls were analyzed using 16S rRNA gene sequencing (V3-V4 region, Illumina NovaSeq). Microbial community profiles were generated with QIIME2, while differential taxa and functional pathways were evaluated using ANCOM-BC and PICRUST2.

Results: *Cutibacterium* and *Staphylococcus* dominated the skin microbiome. While *C. acnes* abundance did not differ significantly between severity groups, moderate-to-severe acne showed higher alpha diversity than controls and mild cases. Specific *C. acnes* IA1 phylotypes were depleted in moderate-to-severe acne and in rural residents. Rural acne patients exhibited higher diversity, distinct community composition, increased environmental taxa (*Kocuria*, *Peptostreptococcaceae*), and reduced *Ralstonia*. Functional predictions showed enrichment of amino acid pathways in urban samples, while rural samples were enriched in amino acid and lipid metabolism but had lower energy metabolism.

Conclusions: Depletion of specific *C. acnes* IA1 strains in association with acne severity is observed in the Egyptian population, independent of lifestyle, suggesting its potential as a biomarker. Emerging therapies should aim to restore microbial diversity and specifically target pathogenic strains while preserving commensal populations.

B184

Utilising an in vitro Gut Model to Investigate Donepezil-Microbiome Interactions

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Abstract

Inter-individual variability in treatment response poses a significant challenge in patient care. The gut microbiome, which varies widely among individuals, affects drug responses. This study aimed to explore how the variable response medicine donepezil impacts the gut microbiome of a healthy volunteer using a reproducible in vitro gut model.

Method: A batch fermentation gut model was optimized to replicate the distal colon environment, considering pH, temperature, and anaerobic conditions. Its functionality was validated through experiments with an untreated negative control, a positive antibiotic control to induce dysbiosis, and donepezil, a variable-response drug. Samples were collected at t = 0, 8, 24, and 48 hours for DNA extraction and 16S rRNA V4 sequencing. Additionally, the growth of gut microbiota isolates was assessed with and without donepezil.

Results: Sequencing results revealed the presence of a diverse microbiome in the simulated models, dominated by the phyla Firmicutes and Bacteroidetes. Notably, a significant change in the microbiome was observed between t=0 and t=8 h, before stabilisation at the 24 h timepoint. Significant changes in beta diversity were observed in microbiome populations by 24 h. The growth of certain gut bacteria isolates was decreased by donepezil.

Conclusion: The gut model employed showed considerable promise as a simulated gut environment for the anaerobic growth and maintenance of gut microbiota. Further optimisation of the model, including timing of drug addition, will improve the robustness of this system as a model for studying the effects of variable response drugs on the gut microbiome.

B185

Bile salts modulate inter-species competition between *Escherichia coli* Nissle 1917 and *Salmonella* Typhimurium

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Abstract

Non-typhoidal *Salmonella* (NTS) is a common cause of self-limiting gastroenteritis, typically transmitted through contaminated food and water, whereas invasive strains (iNTS) can lead to life-threatening systemic infections such as bacteraemia and meningitis. Colonisation resistance mechanisms mediated by the host's intestinal microbiota is an essential defence against *Salmonella* colonisation. This study investigates the mechanisms of inter-species competition between *Escherichia coli*Nissle 1917 (EcN) and *Salmonella* enterica serovar Typhimurium (STm). Two strains were examined: the gastrointestinal strain STm 4/74 (NTS) and the multidrug-resistant, systemically invasive strain D23580 (iNTS). Under anaerobic conditions in LB medium, EcN co-culture with either *Salmonella* strain showed no antagonistic effects. However, addition of 1% (w/v) bile salts caused an approximately 1 Log_{10} reduction in *S*. Typhimurium colony-forming units within 24 hours of interaction when compared with monocultures, indicating that bile salts enhance EcN's competitive advantage. This inhibitory effect required direct cell-to-cell contact and was not mediated by secreted extracellular molecules. Deletion of genes associated with known contact-dependent killing systems including the Type VI secretion system (T6SS), contact-dependent inhibition (CDI), and Type IV pili (T4P) did not abolish the phenotype. These results identify a bile salts enhanced, contact-dependent but uncharacterised mechanism by which EcN inhibits *S*. Typhimurium. Understanding this interaction may inform development of probiotic-based strategies to limit invasive *Salmonella* infections.

B186

Rewilding reshapes gut microbiomes and parasite exposure in European bison: a 17-month release into UK woodland

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Abstract

Reintroductions can restore lost ecological processes, but managers require practical health indicators to track the acclimation of released animals. We longitudinally profiled the gut microbiomes of European bison (*Bison bonasus*) released to the Wilder Blean area (Kent, UK), sampling three adult females before and after release, and a post-release male and calf. Using 16S rRNA gene sequencing, we quantified alpha- and beta-diversity, identified differentially abundant taxa, and screened faeces for *Cryptosporidium*, *Enterocytozoon bieneusi* and *Blastocystis*. Post-release, adult microbiomes shifted significantly (PERMANOVA P = 0.001) with increasing relative abundance of core fibre-associated taxa (e.g. *Burkholderiaceae*, *Methanobrevibacter*) and a decline in *Desulfovibrionaceae* and *Campylobacter*. Calf microbiome profiles transitioned from early-life communities to an adult-like state concurrent with weaning. Parasite screening showed that *Cryptosporidium* positivity declined in females from 36% pre-release to 13% post-release, whereas *E. bieneusi* emerged only after release (~10% of samples), with multiple genotypes detected. These patterns are consistent with dietary and environmental turnover following release, and they highlight opportunities for using microbiome and parasite metrics as complementary, non-invasive indicators of rewilding progress. We recommend reporting simple, management-relevant indicators, archiving sequence data, and documenting soft-release design and supplementary feeding info to aid interpretation. Integrating routine faecal microbiome and parasite monitoring into rewilding programmes can support adaptive management, inform supplementary feeding decisions, and strengthen biosecurity risk assessments.

B187

Co-infection with Streptococcus and Rothia spp. drives prophage dynamics in *Pseudomonas aeruginosa* in an artificial sputum model

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Abstract

Chronic bacterial lung infections with *Pseudomonas aeruginosa* are common in people with cystic fibrosis (CF) but interactions with other commonly seen members of the CF lung community are not well understood. This study aimed to determine the impact of other species on *P. aeruginosa* using an artificial sputum medium (ASM) model designed to mimic CF lung conditions. *P. aeruginosa* LESB58, representing a CF epidemic strain, was inoculated into ASM either alone or in co-culture with *Streptococcus anginosus*, *Streptococcus vestibularis*, *Rothia mucilaginosa* or *Rothia dentocariosa*. Gene expression was assessed using RNAseq at 72 and 96 hours. Free phage numbers were assessed by plaque assay and qPCR. Differential expression of *P. aeruginosa* genes, including those related to virulence factors such as prophage, type III secretion, exopolysaccharides and pyochelin production, was seen in co-culture conditions compared with single-species culture. At 72 h, there was lower expression of most LES prophage genes under co-culture conditions, and this corresponded with lower overall free phage at the same timepoint. During co-culture, there was a reduction in levels of a D3112-like transposable phage, previously shown to have the ability to drive CF-like adaptations in an experimental evolution model, and an increase in levels of Pf1-like filamentous phage, associated with altered biofilm formation and immune responses. Gene expression changes during co-culture with *Streptococcus* and *Rothia* spp. were identified affecting key *P. aeruginosa* virulence factors including phage. This study emphasises the importance of understanding the influence of lung commensals on focal pathogens in a complex microbiome.

B188

Colonization dynamics of *Campylobacter* in the microbiomes of children from the Peruvian Amazon

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Abstract

Diarrheal disease is the third leading cause of mortality in children under five years of age worldwide, with *Campylobacter* as the most common bacterial cause. In low- and middle-income countries, repeated exposure during early childhood is frequent, and up to 10% of children may carry *Campylobacter jejuni* without symptoms. Such asymptomatic carriage has been increasingly associated with growth stunting and impaired cognitive development, highlighting the need to better understand the ecology of persistent *Campylobacter*.

In Iquitos, Peru, enteric infections are highly endemic, and surveillance has reported infection rates of up to 21% among children. Building on over two decades of research for child health and nutrition in the Santa Clara region, we conducted an intensive, community-based study to investigate early-life enteric colonization dynamics. Over a three-month period, fecal samples were collected longitudinally from children under five years, analyzed using both culture-based and qPCR diagnostics, and partitioned for metagenomic sequencing on the Illumina NovaSeq platform to characterize pathogen diversity and subclinical carriage.

Metagenomic data detected *Campylobacter* DNA in samples that were qPCR-negative, indicating frequent low-level colonization below detection thresholds. Sequencing revealed all six ESKAPE pathogens and a consistent core microbiome across 75% of samples, dominated by *Enterobacteriaceae*, *Bacteroidaceae*, and *Bifidobacteriaceae*.

This detailed longitudinal sampling of children in a well-characterized Amazonian community provides rare insight into asymptomatic carriage and its potential role in growth faltering. By combining traditional diagnostics with metagenomic profiling, this study connects microbiological surveillance with the broader context of child health, nutrition, and microbial ecology in an understudied region.

B189

Characterisation of gut bacteriophage diversity and dynamics in preterm infants

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Abstract

The human gut microbiome plays a central role in nutrition, immune development, and host physiology from the earliest stages of life. In preterm infant's (<32 weeks' gestation) gut colonisation is often disrupted by antibiotic exposure, medical interventions, and immature physiology. These disturbances can lead to bacterial dysbiosis and colonisation by multidrug-resistant organisms, contributing to conditions such as necrotising enterocolitis and sepsis.

While bacterial communities in preterm infants have been studied extensively, the gut virome remains poorly characterised. Bacteriophages are major drivers of microbial community structure and function, influencing bacterial fitness, virulence, and antimicrobial resistance. This study aimed to characterise the diversity and dynamics of gut bacteriophages in preterm infants and to compare phage communities between healthy infants and those who developed disease.

A total of 1,874 stool samples were collected from 178 preterm infants in the NICU at the Royal Victoria Infirmary, Newcastle upon Tyne, over the first eight weeks of life. Metagenomic sequencing was performed using Illumina HiSeq X10 (2×150 bp) chemistry. Predicted viral sequences were compiled into a non-redundant custom phage database (3,283 putative bacteriophages, 49.4% >90% complete) and mapped to metagenomic reads from all samples to quantify phage abundance, richness, and diversity. Analysis revealed phage richness and Shannon diversity increased over time, while diseased infants exhibited lower phage abundance but higher diversity than healthy controls. These observations suggest that understanding the early virome is key to uncovering how microbial communities establish and how their disruption may influence disease susceptibility in preterm infants.

B190

Safety assessment of microbiome perturbations

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Abstract

In the consumer goods sector, there is a rapid increase in launches of products that target the human microbiome for cosmetic or health benefits. Currently there are no consensus approaches to assess the possible impact of microbiome perturbations from such products on consumer safety. During a recent academic-industry workshop organised by the Microbiology Society, experts discussed the long-lasting effects and health implications of perturbations induced by products which target the skin, oral and gut microbiomes and how the perturbations should be measured, and their safety assessed. It was concluded that monitoring is necessary to understand potential long-term implications on vulnerable populations. To define risk assessment endpoints, well-designed longitudinal clinical studies need to go beyond taxonomic profiling to include functional microbiome analysis and host-response characterisation. The 3-tier framework we previously developed to qualitatively assess the impact of skin and oral microbiome perturbations on consumer health aligns with the workshop outcomes. Tier 1 benchmarks new actives against products with a proven 'history of safe use'. Tier 2 is based on the notion of 'reversibility of change' as an indicator of microbiome resilience to intervention in clinical studies. Tier 3 aims at ensuring microbiome functions related to host health are not compromised. More research is needed to realise Tier 3 and, in line with the workshop review publication, we conclude that: standardised *in-vitro* models, well documented longitudinal clinical studies integrating different technologies, such as multi-omics and host-response, and data analysis with artificial intelligence will help to improve the safety assessment of microbiome perturbations.

B191

Metagenomic approaches in the detection, surveillance, and clinical management of *Campylobacter sp.* and *Helicobacter pylori*: A systematic literature analysis

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Abstract

Campylobacter sp. and *Helicobacter pylori* are common bacterial gastrointestinal pathogens within the UK; both of which are difficult to culture due to their specific microaerophilic growth requirements, thereby posing potential challenges to clinical management of complex cases or public health surveillance. Metagenomic techniques may offer means for a culture-free alternative to pathogen profiling. To understand how these technologies are currently being applied in this context, a systematic analysis of peer reviewed publications was conducted.

The PubMed database was searched using terms '(Metagenomics) AND ((Helicobacter) OR (Campylobacter))' between March 2015 and June 2025. Resulting records were manually screened to confirm relevancy and remove review or inaccessible papers.

Of 331 results, 56 papers were included based on the study of a pathogen of interest using metagenomic sequencing. Most (48/56) applied either short-read shotgun or, 16s approaches; only 1/56 used exclusively long-read technology. Hybrid approaches were more evident in publications since 2023, with 4/56 studies applying both 16s and short-read shotgun, and 2/56 studies applying both long-read and short-read shotgun sequencing approaches. Overall, Illumina sequencing was the most popular technology of choice. Studies focused on *H. pylori* as pathogen of interest outnumbered *Campylobacter* sp.; and investigated impact on microbiome during infection and treatment, while some of those focused on *Campylobacter sp*. had differing objectives such as identifying reservoirs. Despite some common study limitations, including frequently limited sample sizes, the body of published literature highlights metagenomics as a promising tool for diagnostics and surveillance; although significant barriers remain until routine application is achievable

B192

Multi-omic profiling of oral, gut, and systemic signatures in infective endocarditis

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Abstract

Introduction

Infective endocarditis (IE) is a life-threatening condition with strong but poorly understood links to oral health. To better define this relationship, we investigated the oral and gut microbial communities, and the systemic and salivary metabolome associated with IE to characterise microbial-metabolic interactions across disease stages, from health through high cardiovascular risk to IE.

Methods

Participants included patients with confirmed IE, individuals at high risk according to UK guidelines, and healthy controls (>20 samples per group). Shotgun metagenomic sequencing was performed on saliva and stool samples to profile microbial composition and function (identifying enriched/depleted metabolic reaction pathways). NMR-based metabolomics was applied to saliva and plasma samples to assess local and systemic metabolic alterations.

Results

IE patients displayed marked oral microbial dysbiosis compared to both high-risk and healthy groups. Five microbial taxa were consistently enriched. Network analysis revealed shifts in the microbiome in high-risk individuals, characterised by greater microbial interconnectedness than either healthy controls or IE patients. Similarly, metabolome analysis showed enrichment of key metabolic pathways, including gluconeogenesis, in IE and high-risk groups. Together, these findings suggest that microbial and metabolic shifts precede clinical disease.

Discussion

Distinct microbial and metabolic profiles in saliva, stool and plasma discriminate IE patients, high-risk individuals and healthy controls. These findings provide a new insight into IE development and highlight opportunities for earlier prevention, patient education, and novel risk stratification strategies.

B193

Diversity of the *Bacteroides fragilis* Type VI Secretion System (T6SS)

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Abstract

Bacteroides fragilis is a prevalent member of the gut microbiota. Whilst most strains are commensal, some are enterotoxigenic and associated with colorectal cancer. Humans are invariably colonised by either a commensal or enterotoxigenic strain, but we know little about factors determining which strains successfully colonise. The Type VI Secretion System (T6SS) is an antagonistic weapon used by *B. fragilis* to colonise the gut via interbacterial competition. The T6SS genomic locus contains variable regions encoding for diverse toxic effector proteins, yet how these vary across strains and affect competitive ability is understudied.

We bioinformatically explored T6SS variation in *B. fragilis;* we screened a curated collection of 391 *B. fragilis* genomes for a T6SS locus and grouped loci into variants using sequence similarity clustering. We show that the T6SS is prevalent in *B. fragilis* (~73% of strains), across both commensal and enterotoxigenic strains. Additionally, our study identifies a large number of T6SS variants, with diversity primarily existing in the presence, number and combinations of putative effector and cognate immunity proteins. This prevalence and diversity emphasise the importance of the T6SS as a colonisation factor and suggests a selective pressure acting, which facilitates evolution of the system.

This study provides a large-scale analysis of T6SS variability in *B. fragilis*, providing a foundation to study how variation in the T6SS impacts competitive ability. This work might inform future strategies to rationally prevent or replace enterotoxigenic strains via T6SS-mediated competition.

B194

Isolation and identification of antibiotic resistant bacteria and genes from soil. The effect of animal manure use as a fertiliser on antimicrobial resistance in an Irish beef and sheep farm.

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Abstract

Introduction:

Antimicrobial resistance (AMR) is a public health issue affecting many areas including agriculture. In farms, animal manure can contain antibiotic resistant genes, antibiotic resistant bacteria and undegraded antibiotics. Due to the use of animal manure as a fertiliser and its deposition on soil, there are fears that AMR could spread through the food chain from animals to the environment. Therefore, the impact of animal manure spreading on AMR needs to be investigated.

This study aims to; take soil and animal manure samples before and after animal manure spreading; extract DNA and characterise the taxonomic and AMR gene profile using shotgun sequencing and isolate resistant bacteria to investigate multidrug resistance (MDR) and other resistance mechanisms.

Methods:

Animal manure samples were taken before spreading and soil samples taken before, 2 weeks, 1, 2 and 3 months after manure spreading. Bacterial DNA was extracted from soil and manure and sent for shotgun sequencing.

Antibiotic resistant bacteria were isolated by direct inoculation onto antibiotic-containing media.

Results:

Shotgun sequencing data reflects microbial taxonomy and the presence and persistence of AMR genes in farm soil over a 3-month period.

30 MDR isolated colonies were identified with most belonging to the genus *Pseudomonas*.

Conclusion:

This work investigates the effects of animal manure use as a fertiliser on the spread of AMR on farms. Shotgun sequencing data will reveal the impact of animal manure spreading on the microbial taxonomic and AMR gene profile of soil in agricultural fields.

B195

Patterns in the Unknown

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Abstract

Metagenomics is the study of all microbiota members from a given habitat, where whole genome shotgun approaches sequence all the DNA extracted from those habitat samples. Metagenomic studies often rely on taxonomic classification to describe microbial diversity, however, in some circumstances this approach can introduce significant bias. Alpha diversity (within sample) and Beta diversity (between sample) measures often rely on taxonomy classification at the outset. Traditional taxonomic calls depend on reference databases, that although as comprehensive as possible, still remain incomplete, unevenly annotated, and skewed towards particular taxa and geographical areas. As a result, our view of microbial life is still dominated by known knowns, while the unknown unknowns remain at the edge of our detection. These biases can obscure patterns among the datasets, and lead to incomplete views of community structure and function particularly in highly complex and poorly characterised metagenomes.

Here we describe an alternative approach that focusses on measuring sequence diversity directly, without assigning reads to predefined taxa. This strategy reduces our heavy reliance on reference databases and avoids assumptions about evolutionary relationships or taxonomic boundaries. We have implemented the strategy as a pipeline that results in less biased investigations of diversity across sample datasets and the provision of data visualisations.

By emphasising inherent sequence variation rather than taxa labels, these pipeline methods reveal patterns between communities and can better capture the breadth of microbial diversity present in complex samples.

B196

Evolution of competition in the intestinal microbiome: mechanisms of T6SS effector secretion by gut Bacteroidales

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Abstract

Microorganisms in the intestinal microbiome must compete for resources and space. However, the molecular mechanisms and evolutionary forces underlying these processes are poorly understood.

The type VI secretion system (T6SS) is a macromolecular protein complex found in many microbiota species that mediates intercellular antagonism.

Hemolysin-coregulated proteins (Hcp) are major structural proteins in T6SSs that form hexameric rings that stack to create an inner tube structure essential for translocating toxic effector proteins into target cells.

In Bacteroidales, T6SS loci encode multiple Hcp proteins with unknown functions. For example, the gut commensal *Bacteroides fragilis* encodes five Hcp subunits (sHcp and Hcp1-4) that have low sequence similarity.

In this study, we investigated the roles of these proteins. Biochemical analyses and modelling techniques revealed that sHcp forms the bulk of the inner tube. In contrast, the less abundant minor Hcp1–4 were shown to form an interaction network involving heteromeric complexes.

We also showed that Hcp modules cluster with effectors in regions with conserved synteny. Interaction studies also revealed that minor Hcp heterooligomers recognize effectors to mediate secretion. Moreover, minor Hcps genetically segregate with cognate effectors, contributing to effector cassette variability.

Finally, genomic analyses supported the idea that the gut Bacteroidales are under selective pressure, which drives Hcp-effector cassette evolution and, ultimately, competition among these microorganisms. We discuss the implications for the biodiversity of the gut microbiota and for niche establishment of gut Bacteroidales.

B198

The nematode microbiome: an under-explored source of antimicrobial-producing bacteria

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Abstract

Antimicrobial resistance (AMR) is estimated to cause up to 39 million deaths between 2025 and 2050, with Gram-negative pathogens posing a growing threat to human health worldwide. With the increasing threat of AMR, under-explored areas may offer a novel source of antimicrobials. Nematodes are free-living roundworms and are one of the most abundant animals on Earth, many of which live in close proximity to a diverse ecosystem of microorganisms. These metazoans, and their microbiome, offer a unique, under-explored system that can be utilised in the search for novel antimicrobial-producing bacteria.

Pilot study results have indicated that antimicrobial-producing bacteria are present in the microbiome of nematodes isolated from mosses in Plymouth, UK. Interestingly, they displayed activity against several Gram-negative pathogens.

Following the positive pilot study results, bacterial isolates from the nematode gut microbiome will be screened for antimicrobial activity against a panel of ESKAPE pathogens. Isolates with promising levels of activity will be further screened and identified by 16S rRNA gene sequencing. Additionally, the full expanse of microorganisms found in the moss environments and microbiomes of nematodes will be revealed through 16S rRNA metagenomics.

AMR to clinically available antibiotics is already a concern to human health. Results from this study reveal the microbiome of free-living nematodes as a novel source of antimicrobials, and as a potential contributor to the battle against AMR.

B199

Unravelling the structure and diversity of the healthy human nasal microbiome

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Abstract

Approximately one-third of people carry Staphylococcus aureus in the nose. *S. aureus* carriage is influenced by a range of factors, including host genetics, lifestyle, and interactions with the nasal microbiota. While previous studies have examined the relationship between S. aureus carriage and the microbiota, most have been underpowered and based on 16S rRNA sequencing, limiting taxonomic and functional resolution.

To address this, we established the CARRIAGE study, comprising 20,000 healthy individuals sampled at three time points, one week apart. From this cohort, we generated shotgun metagenomic data for 7,969 samples at the first time point and 430 participants across all three time points. A stringent decontamination pipeline was applied to remove likely contaminants.

We used unsupervised clustering to define community state types (CSTs) within the nasal microbiota. The most influential taxa driving variation were *S. aureus*, *Dolosigranulum pigrum*, *Corynebacterium pseudodiphtheriticum*, and *Corynebacterium accolens*. Our results support earlier findings that intermittent carriers have microbiome profiles resembling either persistent carriers or non-carriers. Strain-level analysis revealed that CST is partly shaped by strain-level differences, with longitudinal data confirming the persistence of both CSTs and specific strains over time. Pangenome analyses of dominant strains are ongoing.

These results advance our understanding of the microbial and strain-level factors associated with S. aureus carriage in the human nose.

B200

Functional bacteriocin diversity drives selective colonisation resistance among commensal Escherichia coli against diarrhoeagenic E. coli

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Abstract

Traveller's diarrhoea (TD) is an enteric dysfunction that frequently occurs during international travel. Enterotoxigenic and Enteroaggregative Escherichia coli (ETEC and EAEC, respectively) cause up to 60% of TD cases. Commensal E. coli can potentially reduce the risk of illness caused by ETEC and EAEC by providing colonisation resistance. One mechanism by which colonisation resistance is achieved is through the production of bacteriocins, short peptides with antimicrobial properties.

Growth inhibition of ETEC (strain: H10407) and EAEC (strain:042) were studied using soft-agar overlays and spotting assays to screen the inhibitory activity of 78 E. coli strains from the human gut. Commensal E. coli genomes were also screened for bacteriocins using BAGEL4. 97% of the genomes contained at least one bacteriocin gene, with 29% containing 5 or more. Carocin D and Colicin E9 were the most common bacteriocins these demonstrating inhibitory activity against 50% of ETEC and EAEC strains. The probiotic strain E. coli Nissle 1917 has 4 bacteriocin loci within its genome, however it displayed no killing activity against ETEC or EAEC. Out of 82 E. coli strains tested, only 12 showed consistent killing activity against ETEC and EAEC.

ETEC and EAEC displayed similar susceptibility profiles to bacteriocin-producing E. coli strains. Together these findings suggest that bacteriocin-mediated killing of E. coli is dependant on genomic context but is a valid strategy to provide colonisation resistance to ETEC and EAEC.

B201

The impact of *Wolbachia*-infection on the microbiome in *Culex quinquefasciatus* dissected midguts.

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Abstract

Culex quinquefasciatus mosquitoes are found in a number of tropical and sub-tropical regions globally and can be vectors of parasites and viruses including avian malaria and West Nile virus. Culex mosquitoes are commonly infected with *Wolbachia*, a genus of obligate-intracellular, endosymbiotic bacteria that infect roughly 60% of arthropod species. *Wolbachia* are of interest due to the potential capability of preventing pathogen replication within the host mosquito.

The aim of this study was to assess the impact of natural and transinfected *Wolbachia* infections on the host microbiome composition and diversity in Culex quinquefasciatus mosquitoes. This was achieved by assessing three *Culex* lines. The first line was naturally infected with *w*Pip *Wolbachia* strain, the second was aposymbiotic tetracycline cured line, and the final was transinfected with wAlbB *Wolbachia* strain. Dissected midguts from each condition were subjected to Oxford Nanopore sequencing while the V3-V4 hypervariable regions of the 16S rRNA gene was sequenced using the Illumina platform. This also enabled a direct comparison of both sequencing methodologies.

No significant difference was found in the composition of the microbiome between the naturally infected with *Wolbachia* and transinfected lines. There was a higher relative abundance of *Wolbachia* in the midgut of the transinfected line compared to the naturally infected line. Some key taxa driving differences observed between lines include Cedecea, *Enterobacter*, *Aeromonas* and *Delftia*. Nanopore sequencing was able to capture more significant differences in alpha diversity between conditions than was seen in Illumina amplicon sequencing data.

B202

Characterising the impact of viral infection on the respiratory tract microbiome using a multi-omics approach.

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Abstract

The interactions between respiratory viruses and the respiratory tract microbiome is understudied, especially how commensal microbes protect against invading viruses. This project investigates the dynamics between commensal microorganisms and common viral infections, focusing on shifts induced by viral infection in individuals of typical and non-typical microbiomes. Leveraging a biobank of over 28,000 nose/throat swab samples from the UK HSA Lighthouse Labs, we will compare the microbiome diversity in infected and uninfected individuals. Concurrently, we will investigate those with inflammatory bowel disease (IBD), a condition associated with severe viral illness and altered microbiomes, using a separate over 400 respiratory swab sample biobank from Birmingham DRTB, taken from IBD patients and non-IBD controls. We will employ a multi-omics strategy with a sequencing-based approach to profile bacterial composition and transcriptomics, high-throughput microscopy for viral diversity, and mass spectrometry to assess shifts in protein expression. The data will provide information for commensal bacterial shifts along with gene and protein expression changes. Protein candidates derived from the respiratory microbiome, both those newly discovered from our analysis and existing candidates identified from the literature, will be purified and tested for antiviral efficacy against respiratory viruses (e.g. influenza) using techniques like plaque assays. Ultimately, identifying microbial-derived inhibitors or activators of viral infection could lead to new therapies targeting viral infections as well as restoring microbiome balance in individuals with non-typical microbiomes.

B204

Breastfeeding and the milk resistome shape the establishment and transmission of antibiotic resistance genes in the infant gut microbiome

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Abstract

The infant resistome, the collection of antibiotic resistance genes (ARGs) of newborns, is critical for gut microbiota establishment. Using metagenomic sequencing data, we analyzed various 1-week and 1-month postpartum samples to study infant resistome establishment, ARG transmission, and its impact on functional redundancy of the microbiota. A total of 431 samples were analyzed; infant stools (1-week, *n* = 119; 1-month, *n* = 119), maternal stools (1-month postpartum, *n* = 120), and breastmilk (1-month postpartum, *n* = 73). Breastfeeding correlated with increased functional redundancy and altered bacterial-ARG co-occurrence networks in the infant resistome. *Escherichia coli* dominated early resistome dynamics with a higher abundance correlating with reduced functional redundancy. *Bifidobacterium longum* exhibited a consistent negative association with 21 ARGs at one-month in breastfed infants, while four negative relationships between ARGs and *Bifidobacterium bifidum* were observed in formula-fed infants. ARG transmission via breastmilk appears to be gene-specific, with the quinolone resistance gene *sdrM* likely transmitted under maternal antibiotic use. Delivery mode modulated the microbial environment in ways that interact with resistome structure and changing functional redundancy, particularly through genera like *Staphylococcus* and *Streptococcus*. These findings highlight the role of early feeding practices in resistome development and propose functional redundancy as a key ecological framework for understanding infant gut resistome dynamics.

B205

Improved Pathogen Detection in Catheter-Associated Urinary Tract Infections (CAUTI) Using Nanopore Metagenomic Sequencing

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Abstract

Catheter-associated urinary tract infections (CAUTIs) remain the most common hospital-acquired infection and a major driver of unnecessary antibiotic use. Conventional urine cultures often yield false negatives, particularly after empirical therapy, or ambiguous “mixed growth” results. Therefore, comprehensive pathogen identification to guide targeted therapy and reduce antimicrobial misuse is urgently needed.

We evaluated nanopore metagenomic sequencing as a diagnostic adjunct for CAUTI by analysing urine samples from catheterised patients and comparing results with routine microbiology tests. Nanopore sequencing and standard culture were concordant in 63% of cases. In the remaining 37%, metagenomics provided additional or refined diagnostic information: in 26% of samples, clinically relevant organisms were identified that were missed by culture or revealed closely related but distinct taxa. In 11%, metagenomic analysis clarified samples reported as “heavy mixed growth”, providing clearer pathogen definition and diagnostic insight.

Overall, nanopore sequencing identified polymicrobial infection, improving pathogen identification in every case. The workflow combined physical depletion of human cells with taxonomic classification using Kraken2 and downstream genome coverage and distribution assessment, enabling comprehensive pathogen characterisation directly from urine samples. Median sequencing output comprised 39,451 bacterial reads (IQR 25,112–81,821) and 214,496 host reads (IQR 80,602–370,909).

These findings show that nanopore metagenomic sequencing can enhance diagnostic accuracy and pathogen resolution in CAUTI. Integrating this approach into clinical workflows could enable more targeted antimicrobial therapy, improve patient outcomes, and reduce inappropriate antibiotic exposure.

B206

Characterising the impact of antibiotics used to treat respiratory infections and modulator therapy drugs upon the gut microbiome of children with cystic fibrosis using a high-throughput *in vitro* gut models.

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Abstract

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations to the cystic fibrosis transmembrane conductance regulator (CFTR) gene, estimated to affect over 160,000 people worldwide. People with cystic fibrosis (pwCF) experience systemic implications due to mutations in the CFTR gene, more locally in the gastrointestinal (GI) tract, this manifests as alterations in mucus viscosity, motility and pH.

The gut microbiota of pwCF is characterised by a delayed maturation, significantly reduced microbial diversity and an altered composition characterised by an increased abundance of *Enterococcus*, *Veillonella*, and *Streptococcus* species, alongside a decreased abundance of *Bifidobacterium*, *Roseburia*, and *Alistipes* species.

The use of novel CFTR modulator drugs partially restores function to the mutated CFTR protein, which is thought to improve GI physiology. However, the gut microbiota remains distinct from that of healthy individuals. Persistent antibiotic use in pwCF is a likely contributor to this discrepancy, yet its effects on the gut microbiome have not been comprehensively investigated.

To address this, two 96-well plate *in vitro* gut models resembling the healthy physiology (MiPro) and CF physiology (CF-MiPro) will be used as a powerful high-throughput tool for characterising drug-microbiome interactions. This approach will enable detailed genomic and metabolomic analyses to elucidate the effect of antibiotics on the structure and function of the gut microbiota in pwCF.

B207

Characterising Strain-Level Diversity of Wound versus Healthy Skin Staphylococcus Isolates Using a Human-Relevant Wound Model

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Abstract

Chronic wounds cost the NHS £8.2 billion annually, and bacterial infections precede 90% of amputations. Our group first demonstrated skin microbiome as an important healing outcome predictor in chronic wounds. However, our understanding of their mechanisms is limited due to poor translation of murine models and lack of *in-vitro* models fully representing human skin microbiome. We address this key knowledge gap by developing a human *ex-vivo* skin microbiome platform to dissect the crucial role of bacteria in wound pathology.

We have curated a biobank of over 300 *Staphylococcus* strains from healthy skin and wound infections. We compared biofilm forming capabilities, antibiotic resistance and haemolytic activity. Each characteristic was graded; isolates with highest and lowest grades (most to least virulent) were selected for testing strain diversity in our human *ex-vivo* wound model.

Our preliminary data reveals strains of same species varying distinctly in biofilm formation, antibiotic resistance, and haemolytic potential. Notably, enhanced biofilm formation was observed under low pH and high glucose conditions. Antibiotic resistance was distributed primarily across S. epidermidis and S. aureus, with strain-specific resistance patterns. Strain-level functional variations were evident in our ex-vivo wound model, with divergent effects on wound closure rates, linked to bacterial virulence and isolation site. Ongoing long-read whole genome sequencing analysis is identifying genomic variations within strains, which will link to donor metadata, phenotypic characteristics and wound healing outcomes.

These findings demonstrate our ex-vivo model as predictive for bacterial pathogenicity screening and highlight importance of strain-level analysis in understanding microbe-host interactions in wound healing.

B208

Revealing the microbial dynamics which facilitate successful ruminant methane mitigation *via* novel oxygen-releasing feed additives

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Abstract

Enteric methane (CH_4) emissions from ruminant livestock greatly contribute to global CH_4 production, mitigation of which is essential to slow the impact of climate change. Ruminant feed additives offer a cost-effective means of accomplishing this. Previous research showed that minor elevations to the rumen oxidation reduction potential (ORP) using oxygen-releasing compounds can hinder enteric methanogenesis, given that ruminant methanogens are typically only active at ORPs below -300 millivolts. *In-vitro* assessment of these compounds, including liquid hydrogen peroxide (H_2O_2); encapsulated liquid H_2O_2 for controlled, slow release; and both calcium and magnesium peroxide (CaO_2, MgO_2), has demonstrated effective CH_4 mitigation potential, with consistent CH_4 reductions of >50% observed. An encapsulated format of MgO_2 and CaO_2 could offer feasibility as a CH_4 mitigation feed additive solution in both pasture-based and intensive production systems. However, the influence these oxygen-releasing compounds have on the rumen microbiome requires deeper investigation. This study establishes the *in-vitro* influence of ORP modulating compounds on rumen microbial communities. Following co-extraction of Nucleic Acids from rumen fluid, amplicon sequencing of the 16S, 28S and ITS genes was performed on all samples (n=64). Results revealed clear microbiome shifts away from common methanogenic pathways, whereby significant reductions in archaeal species richness and diversity was associated with all compounds to varying extents, with no negative impacts on digestibility of feed from MgO_2 and encapsulated liquid H_2O_2. A clearer understanding of the these microbiome impacts will facilitate successful translation of a ruminant feed additive towards large-scale application on farm.

Session : Pathogens without borders: Multi-host veterinary infections

B209

Rhizospheric-dwelling Salmonella enables cross-kingdom invasion by flagella-driven motility and EPS-mediated attachment to organic matter

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Abstract

Salmonella is an established pathogen of the members of the kingdom Animalia. Reports indicate that the association of *Salmonella* with fresh, edible plant products occurs at the pre-harvest state, i.e., in the field. In this study, we follow the interaction of *Salmonella* Typhimurium with the model plant *Arabidopsis thaliana* to understand the process of migration in soil. Plant factors like root exudates serve as a chemoattractant. Our ex-situ experiments allowed us to track *Salmonella* from its free-living state to the endophytic state. We found that genes encoding two-component systems (TCS) and proteins producing extracellular polymeric substances (EPS) are essential for Salmonella to adhere to the soil and roots. To understand the trans-kingdom flow of *Salmonella*, we fed the contaminated plants to a murine model and observed that it invades and colonizes the liver and spleen. To complete the disease cycle, we re-established the infection in the plant by mixing the potting mixture with the fecal matter collected from the diseased animals. Our experiments revealed a cross-kingdom invasion by the pathogen via passage through a murine intermediate, a mechanism for its persistence in the soil, and invasion in a non-canonical host. These results form a basis to break the life-cycle of *Salmonella* before it reaches its animal host and thus reduce *Salmonella* contamination of food products.

B210

Comparative Analysis of the Mobilome and Associated Virulence Factors in Salmonella Dublin from Different Hosts

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Abstract

Salmonella Dublin (SD) is a zoonotic pathogen causing invasive disease. It causes bacteraemia and meningoencephalitis with high mortality and abortions in cows. Asymptomatic carriage can occur in older animals leading to shedding and transmission within herds, complicating surveillance and eradication. Human transmission can occur via consumption of contaminated food and direct contact with infected animals, generally causing disease with higher fatality rates than other serovars. Despite this, relatively little is known about SD. The aim of this study was to perform an in-depth genomic characterisation of SD to identify virulence factors and zoonotic traits. We interrogated public sequence data available in Enterobase using ABRICATE (vfdb, Resfinder, PlasmidFinder) and Roary to identify genomic markers in either cattle (n = 2485) or human associated (n = 1212) isolates. We found *S*. Dublin to be a highly clonal serovar with a closed chromosomal pangenome. We identified 5560 plasmids with multiple incompatibility groups. Most isolates contained at least one plasmid with *spv* virulence genes. The plasmids harboured the majority of AMR genes found, conferring resistance to most antibiotic classes. Chromosomal virulence genes were found on *Salmonella* Pathogenicity Islands (SPI-1 and SPI-2) and prophages (Gifsy2). Notably, this included SPI-7 containing the *S*. Typhi Vi capsule in 38 isolates. The data allow us to link genomic plasticity to carriage and transmission risk across hosts and to understand the mobility of virulence and AMR elements, which is critical to enable threat prediction and control of *S.* Dublin across populations and to support broader economic and food security.

B211

Investigating the Mechanisms of *Salmonella* Dublin Persistence in Bile

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Abstract

S. Dublin is a host-restricted serovar of *Salmonella enterica* subspecies *enterica.* It is the most-commonly isolated serovar from cattle and causes systemic infections resulting in bacteraemia, meningoencephalitis, high calf mortality and abortions in pregnant cattle, which has significant impacts on the dairy and beef industries. *S.* Dublin can be transmitted to humans by direct contact with infected animals and the consumption of contaminated foods. Although the incidence is low, *S.* Dublin is associated with high mortality in humans. In cattle, *S.* Dublin can persist asymptomatically in the gallbladder once symptoms resolve. From here, it can re-enter the intestine and be shed in faeces leading to undetected transmission within the herd. The gallbladder is the site of storage for bile, which can be bactericidal at high concentrations. To investigate how *S.* Dublin can persist in this niche, a high-throughput mutagenesis and sequencing method called transposon-directed insertion site sequencing (TraDIS) will be applied. TraDIS libraries will be generated in well characterised strains of *S.* Dublin and screened for survival in 10% ox-bile to mimic gallbladder conditions. Sequencing of mutant libraries before and after culture in bile will identify genes required for luminal gallbladder survival. Alongside this, *S.* Dublin will be passaged long-term in 10% ox-bile to determine whether genome rearrangement facilitates gallbladder persistence by impacting fitness and virulence. Together, these studies will improve our understanding of how *S.* Dublin persists in the gallbladder and offer opportunities to develop therapeutic targets to reduce asymptomatic carriage, improve bovine health and prevent zoonoses.

B212

Mycobacterium bovis genomic epidemiology provides evidence on cross border cattle trade and deer risk in Ireland?

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Abstract

Background

Bovine tuberculosis incidence in Ireland is at an historic 15-20 high with herd level figures in Northern Ireland (NI) at ~11% and in the Republic of Ireland (RoI), ~6%. Policy makers in both jurisdictions have expressed concern on the role deer may be playing in disease epidemiology and whether cross border trade in cattle is contributing to persistence and transmission in 'hotspots' in border Counties. We undertook local genome epidemiological investigations to inform the policy response.

Methods

We sampled bTB outbreaks across the Irish border in Counties Monaghan, Armagh, Tyrone and Fermanagh to construct a dataset of 481 *Mycobacterium bovis* genomes. We also undertook a NI wide survey of wild and peri-domesticated deer to assess disease prevalence and attain cultures of *M. bovis* from affected cervids. We sampled cattle derived isolates from AFBI's archive exhibiting close genetic relatedness and sympatry to those derived from deer to construct a dataset of 207 *M. bovis* genomes. We applied structured coalescent phylodynamic models to both datasets to inform on transmission dynamics.

Results

Cross border analyses indicated that most disease transmission events were occurring within NI and RoI territories. Inter-territorial events were rarer and equivalent in magnitude between NI and RoI, suggesting that cross-border control measures were more effective compared to standard testing protocols applied within individual jurisdictions.

Deer and bovine analyses indicated that bTB deer prevalence was ~2.5% and that inter-species transmission events were likely occurring and equivalent in magnitude. However, most of the disease transmission was observed to be from cattle-to-cattle.

B213

Investigating phenotypic variation within *Salmonella* Dublin

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Abstract

Salmonella Dublin is a bovine host-restricted serovar of *S. enterica* that occasionally causes disease in humans and other livestock. In the UK, it was one of the most common serovars isolated from cattle annually since 2013. S. Dublin causes invasive disease with high mortality in calves and can result in abortions in pregnant cows. It can be carried asymptomatically in older cattle, complicating surveillance and eradication. It also has a high invasive index in humans and is associated with higher hospitalisation and mortality rates than other non-typhoidal *Salmonella* serovars. Although a significant zoonotic pathogen, *S.* Dublin remains relatively understudied. Here, we are performing a large-scale systematic phenotypic screen of disease-causing isolates from cattle and humans to better understand the variation in virulence within this serovar. Thus far, differences have been observed in the growth of isolates in lysogeny broth and lysogeny broth supplemented with a high concentration of ox-bile to mimic the bovine gallbladder, where *S.* Dublin can persist. Moreover, isolates have been found to vary greatly in swimming and swarming motility, abilities that are required for colonising host tissues and which also correlate with the inflammatory potential of isolates owing to the immunogenicity of flagella. Further testing of these isolates to assess other virulence traits, including biofilm formation, invasion, and survival in host cells from cattle and humans, will improve our understanding of the virulence of S. Dublin, which in turn will benefit the development of public health surveillance and intervention strategies.

B214

Industrial Chicken Farming Reshapes the Evolutionary Dynamics of Campylobacter jejuni

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Abstract

Since 1960, the global chicken population has increased 7 fold. As a major campylobacter host this has massively altered *Campylobacter jejuni* populations, changing both population structures and host associations.

We used phylogenetic methods and genome-wide association studies to dissect these changes, focusing on three questions: (1) how clonal complexes partition across hosts, (2) which genetic features are enriched in chicken-associated isolates compared to wild birds, and (3) how strain population sizes and host transition rates have shifted over time.

Our analyses reveal clear contrasts in host ecology. Certain lineages remain highly specialised for wild birds, while others have more generalist lifestyles. Chicken-derived isolates show distinct genetic signatures, pointing to adaptation to poultry farming environments. Crucially, we find evidence of rapid population growth among chicken-associated strains and an increase in host switching events that correlate with the rise of intensive poultry production.

Overall, these results reveal how intensive animal farming can change a pathogens evolutionary landscape, increasing the frequency of host transitions and recombination events.

B215

Hepatitis E virus surveillance study in Northern Ireland pig herds and other animal reservoirs

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Abstract

Background

Hepatitis E virus (HEV) is an emerging global pathogen and a causative agent of a viral hepatitis disease, where pigs are a known viral reservoir. Zoonotic transmission occurs via the oral-faecal or food-borne routes of infection. People who work directly with pigs, consume raw or under cooked pork products and immunocomprised people are most at risk of HEV infection. Limited information exists on HEV epidemiological surveillance studies especially when considering HEV animal reservoirs and transmission links from 'farm to fork.'

Methods

We established the prevalence of HEV in the NI pig population by testing HEV IgG antibody levels (*n*=448 sera) and HEV RNA viral load by qPCR from a pig abattoir biobank (*n*=960 liver, sera and faeces). Additional biobanks tested included pig, mixed livestock and wildlife samples submitted to AFBI for postmortem.

A molecular epidemiological longitudinal study of six farms was conducted by tracking 25 pigs per farm for 20-weeks at five time-points to identify HEV infection dynamics and viral shedding (*n*=750). Co-factor infections were detected and metadata collated. A d*e novo* viral metagenomics approach was used to genotype and phylogenetically analyse the NI HEV.

Findings

The NI pig abattoir HEV serological prevalence survey result was 52.46% or 91% positivity at farm level. Although viral RNA detection levels were low, concerningly, positive pig samples indicated high viral loads. Interestingly, HEV was detected in other livestock whereas wildlife samples were negative. All longitudinal farms detected HEV at each time-point but demonstrated different infection dynamics and even revealed a reinfection occurrence.

B216

Restricted gene flow and loss of core gene function accompanied the emergence of a host-specialist lineage of Staphylococcus aureus

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Abstract

Staphylococcus aureus is a multi-host bacterial pathogen that causes a variety of globally important human and animal diseases. The *S. aureus* species pangenome is defined as open, consistent with high frequency of horizontal gene transfer across the species. Consistent with this, adaptation of *S. aureus* to different hosts is mediated in part by lineage-specific sets of horizontally acquired accessory genes, often associated with mobile genetic elements (MGE).

We have discovered that intra-species lineages of *S. aureus* (represented by clonal complexes; CCs) exhibit extensive variation in pangenome openness, that correlates with the size of their known host-species range. Of note, the major ruminant-specific *S. aureus* lineage CC151 has a very closed pangenome, indicative of a conserved and widely distributed complement of MGE. Furthermore, we have discovered that CC151 has shorter genomes than average, which is correlated with a conserved mobilome and barriers to the acquisition of new MGEs. CC151 genomes also demonstrate an abundance of truncated core genes relative to other lineages. Phylogenetic analysis indicates that CC151 lost the function of some genes very early in its evolution, including a proportion predicted to be involved in direct interactions with the host.

Together, our analysis provides a detailed case study of the genetic basis for the emergence of host-specialised lineages within generalist bacterial populations.

B218

Exploration of genotypic diversity and risk assessment of ruminant-associated *Coxiella burnetii* in Great Britain using MLVA genotyping

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Abstract

Coxiella burnetii is a Gram-negative, obligate intracellular zoonotic bacterium that infects a wide range of hosts, including humans. Cattle and small ruminants are recognised as reservoirs for human infection. In the UK, Q fever is notifiable in humans under the Health Protection Regulations (2010), and since 2021, detection of *C. burnetii* in animals has been reportable across GB and the EU. Despite its widespread distribution, the molecular epidemiology of *C. burnetii* in UK livestock remains poorly characterised.

This study aimed to characterise *C. burnetii* strains circulating in Great Britain using Multiple Locus Variable-number Tandem Repeat Analysis (MLVA). MLVA typing was performed on 51 samples (30 cattle, 4 sheep, 17 goats) submitted to APHA between 2013 – 2024 that were confirmed *C. burnetii* positive by qPCR (Ct range: 10.52–23.18) and diagnostic protocols. MLVA targeted six loci (MS23, MS24, MS27, MS28, MS33, and MS34) and initial validation of PCR assays using *C. burnetii*Nine Mile reference DNA produced the expected MLVA typing profile.

MLVA typing was applied to all isolates and *In silico* analyses were performed to assign an MLVA genotype to each *C. burnetii* isolate. The ability to obtain MLVA genotypes was assessed relative to the abundance of *C. burnetii,* as determined by *IS1111* qPCR, and types were compared across animal species, the reference strain Nine Mile genome, and reference datasets. Initial analysis indicates the presence of multiple genotypes in the UK. These findings will improve understanding of *C. burnetii* genetic diversity in Great Britain and inform future epidemiological investigations.

B220

Using a Life-Identification Number (LIN) coding system to improve knowledge of Campylobacter transmission at multiple levels of resolution

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Abstract

Cases of human Campylobacteriosis, many of which are resistant to antimicrobials, continue unabated worldwide. Therefore, further elucidation of transmission routes is crucial. Molecular typing systems using multilocus sequence typing (MLST) have improved knowledge of host associations by the organism. However, higher-resolution, core genome MLST (cgMLST) nomenclature used for fine typing is unstable. We have developed a new joint *C. jejuni* and *C. coli* typing scheme, Life Identification Number (LIN) codes, that is scalable and compatible with existing nomenclature.

A subset of 5,664 high-quality genomes (5,224 C. jejuni and 440 *C. coli*) were randomly selected from the PubMLST Campylobacter database, covering a broad range of hosts, geographical regions, and time periods. A distance matrix was generated using the PubMLST Genome Comparator tool, and dissimilarity thresholds were calculated. Eighteen LIN thresholds were identified to represent a multi-level nomenclature that is now openly available from PubMLST.

To ensure LIN code integrity, two recombination-adjusted maximum likelihood phylogenies were created using 1,633 and 118 *C. jejuni* and *C. coli* genomes, respectively. LIN-defined groups were largely congruent, with monophyletic clades in the tree. The LIN codes also resolved paraphyletic ccs (ie cc692) and other sub-structures (ie cc21) within *C. jejuni* .

We validated the LIN codes using isolates from a mixed-source, water-related outbreak in New Zealand to assess epidemiological resolution. This highlighted connections between sources at high-resolution. Overall, our unified framework bridges population genetics and applied surveillance, enabling robust, real-time comparison of *Campylobacter* isolates across sources, studies, and time.

B221

Prophage-mediated avian host-specific pathogenesis of *Staphylococcus aureus*

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Abstract

Staphylococcus aureus has a remarkable capacity to adapt to different host species, leading to emergence of host-specific pathogenic lineages. In particular, *S. aureus* causes skin and joint infections in poultry responsible for considerable economic and welfare impact on the broiler industry. We have previously determined that an avian-specific sub-lineage of the global human sequence type 5 *S. aureus* evolved via a human-to-chicken host jump event followed by host adaptation via gene acquisition, including an avian Sa3 beta-converting prophage (ϕAvβ). Here, we examined the diversity of beta-converting phages across the diversity of human and avian *S. aureus* and evaluated the contribution of ϕAvβ to avian host-*S. aureus* interactions. Distinct variants of ϕAvβ were identified in multiple avian-associated lineages including hybrid phages that resulted from recombination events associated with replacement of the human Immune Evasion Complex with avian-specific genes. Importantly, generation of ϕAvβ-deletion mutants revealed decreased survival in chicken plasma and whole-blood compared to ϕAvβ-positive parent strain, independently of beta-toxin. Furthermore, mutants displayed impaired growth in the presence of hydrogen peroxide. Overall, our results indicate that ϕAvβ has spread across the avian *S. aureus* phylogeny, promoting survival against the avian immune system. We have now developed a genome-wide transposon library in an avian *S. aureus* strain background which is being employed in a directed screen in chicken whole-blood to identify prophage-encoded and other bacterial effectors of avian host-adaptation. These data are providing new insights into bacterial host-adaptation that may inform the design of novel approaches for control of avian *S. aureus* infection.

B222

Impact of a circular economy protein source on poultry zoonotic pathogen load.

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Abstract

Dietary protein source and quality can influence the gut microbiome and significantly affect the load of zoonotic pathogens such as Salmonella, Campylobacter and Clostridium perfringens, posing potential food safety risks. This study investigated the effects of exposure level and prior experience with a bacterial Single Cell Protein (SCP) supplemented diet on zoonotic pathogen load in poultry. Birds were fed diets containing SCP at two inclusion levels (6.25% or 12.5%), with or without prior exposure (0% or 3.125%), in a 2x2 experimental design. Caecal samples from 21- and 35-day old birds were cultured for C. perfringens, Salmonella and Campylobacter, with identification by MALDI-TOF MS. Results obtained showed absence of Salmonella, while Klebsiella pneumoniae, Proteus mirabilis, Shigella spp and Escherichia spp were present in samples of 35 days of age. Proteus mirabilis counts were highest in Groups 1 and 2 (3.72×10^8 CFU/mL) and lower in Group 4 (1.20×10^7 CFU/mL), with no isolates in Groups 3, 5, and 6. Escherichia spp. showed greatest abundance in Group 4 (7.0×10^{10} CFU/mL), followed by Group 5 (3.68×10^4 CFU/mL), while Klebsiella pneumoniae occurred only in Group 4 (1.20×10^{12} CFU/mL). Shigella spp. appeared solely in Group 2 (8.0×10^7 CFU/mL). mL). Enterococcus faecium and Enterococcus gallinarum were frequently isolated, while Clostridioides difficile was detected at low levels ($\approx 1.1 \times 10^5$ CFU/g). Campylobacter spp were rare, occurring mainly in group 4. These findings indicate that SCP inclusion modulated caecal bacterial composition, with shifts observed among opportunistic species.

Session : Plant-Pathogen Interactions

B223

High-Resolution Mapping of Bacterial Colonisation of Arabidopsis Roots with Fourier Ptychographic Microscopy.

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Abstract

The model species of *Arabidopsis* is well established within the field of botany to study interactions between plants and micro-organisms. Imaging of these interactions is usually performed in light microscopy with diffraction-limited methods. However, these approaches can image either a small field of view at high resolution or a large field of view at low resolution. As such, imaging of bacterial colonies along plant roots cannot be resolved in sufficient numbers for meaningful statistical interpretation.

Fourier Ptychographic Microscopy (FPM) combines known properties of Fourier optics with ptychographic imaging and is a low-cost solution for high resolution imaging over large areas. By using a low magnification objective lens and an LED array, FPM varies illumination angles to capture a sequence of images and a reconstruction algorithm combines them into a single high-resolution image. This results in detailed intensity and phase images from original low-resolution datasets. Since its first use, FPM has been primarily focused on improvements to the computational reconstruction and phase retrieval rather than a tool for biological research.

We have, for the first time, applied FPM for label-free imaging of *Pseudomonas aeruginosa* infection of *Arabidopsis* roots. By training a machine learning network, we have shown that it is possible to identify the pathogen separately from the host. With these new approaches, we aim to identify colonisation patterns to better understand plant-pathogen interactions with our improved resolution over a large field of view.

Session : Transformative tools for biological complexity

B224

SEISMIC: Uncovering cellular and subcellular heterogeneity with multi-omics

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Abstract

Until now, conventional bulk measurements have dominated the study of biological processes. While informative about overall cellular composition and function, these approaches obscure critical differences between individual cells and organelles, masking cellular heterogeneity - even though such heterogeneity can shape infection outcomes, drug responses and metabolic adaptation. Conventional mass spectrometry imaging and biochemical assays also often require chemical fixation or embedding, which can delocalise analytes, disrupt physiological states, and compromise organelle integrity.

To address this challenge, the SEISMIC facility for single cell and subcellular omics integrates fluorescence imaging, targeted nanocapillary extraction, and high-sensitivity mass spectrometry to analyse whole live cells and subcellular compartments - including cytosol, mitochondria, nucleoli, stress granules, lipid droplets, and intracellular pathogens - without fixation. Central to this approach is the Single Cellome Yokogawa SS2000, an automated system that isolates live single cells in nano capillary tips with minimal perturbation, preserving both viability and spatial information.

SEISMIC has already provided novel insights into cellular and subcellular dynamics, with applications including intracellular bacterial sampling, drug quantification in single cells, lipidomic profiling of x-ray–irradiated cancer cells, metallomic analysis for radiopharmaceutical tracking, and determination of metabolic differences between BCG-infected cells and bystanders.

By enabling profiling across multiple molecular classes - lipids, metabolites, proteins, drugs, and metals - SEISMIC captures the full complexity of cellular states. This integrated platform opens new avenues for investigating organelle-specific functions, dynamic cellular adaptations, and host-pathogen interactions. By preserving live-cell context, SEISMIC is redefining the study of biological heterogeneity and unlocking dimensions of cell biology previously considered inaccessible.

Session : Virus Forum: Molecular & Cellular Biology

B226

Stress Granule Dynamics Shape Influenza A Virus Replication at Lower Temperatures

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Abstract

This study investigates the relationship between stress granule formation and the replication dynamics of influenza A virus (IAV) at different physiological temperatures. Stress granules (SGs) are dynamic, non-membranous ribonucleoprotein biocondensates that form in response to cellular stresses, including viral infection and temperature fluctuations. These granules play a key role in regulating mRNA translation and innate immune responses. This investigation assesses whether altered SG assembly modulates viral replication efficiency, or conversely, whether IAV itself actively influences SG dynamics and host protein localisation at different physiological temperatures. Using human epithelial cells exposed to physiological (37 °C) and sub-physiological (33 °C) temperatures, reflective of the upper and lower respiratory tract, we quantified SG formation following both chemical induction and IAV infection. Immunofluorescence analyses were employed to visualise SG markers such as G3BP1, while viral replication was evaluated through quantitative PCR and plaque assays. Preliminary results suggest that SG formation is increased at lower temperatures. IAV mRNA is visualised to localise within these granules, suggesting that IAV may hijack these SGs during replication. The specific host and viral mRNAs associated with stress granules remain unidentified, limiting our understanding of how influenza A virus may utilise these structures to support replication under lower-temperature conditions. These potential findings highlight a reciprocal relationship between temperature, SG dynamics, and viral propagation. Understanding how environmental factors such as temperature influence stress granule biology and virus-host interactions may provide new insights into influenza pathogenesis and transmission, particularly in the context of seasonal variability and respiratory tract localisation.

B227

Dissecting mechanisms of Orthoflavivirus translation

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Abstract

Viruses, including members of the *Flaviviridae* family, are obligate intracellular parasites, and therefore must rely on the host translational machinery to replicate.

The 5′ cap is a conserved feature of eukaryotic mRNAs, essential for cap-dependent translation initiation. Multiple cap structures exist depending on their methylation patterns: Cap 0 has a 7-methylguanosine, promoting efficient translation, while Cap 1 and Cap 2 include additional 2′-O-methylation on cap-proximal nucleotides preventing innate sensing and further enhancing translation.

Orthoflaviviruses produce cap 1 structures on their mRNA, through non-structural proteins, NS3- and NS5-mediated enzymatic steps, including removal of the γ-phosphate, addition of GMP via a 5′–5′ triphosphate linkage, and methylation at the N7 (Cap 0) and 2′-O (Cap 1) positions. This suggests their translation during infection occurs via canonical cap-dependent initiation, however, opposing evidence exists. Currently no consensus has been reached regarding the exact mechanism(s) of potential cap-independent translation utilised by *Orthoflavivirus* genomes. Here, we aim to test alternative mechanisms in the context of *Orthoflavivirus* translation, and identify novel protein interactions which may aid these alternative mechanisms.

Using *in vitro* and cell-based reporter assays, we further investigate the molecular mechanism(s) behind *Orthoflavivirus* translation. In agreement with previous literature, we find that the translation of different orthoflaviviruses is maintained when cap-binding protein eukaryotic initiation factor 4E (eIF4E), a key regulator of cellular translation, is sequestered. Using a proteomics approach, we identify multiple novel RNA-protein interactions with the *Orthoflavivirus* 5′UTR that we will investigate for effects on translation efficiency during cellular stress conditions.

B228

Characterisation of VHS endoribonuclease variants that spontaneously arise in VP22 deletion viruses of herpes simplex virus 1.

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Abstract

Herpes simplex virus 1 (HSV1) exhibits a cascade of gene expression, culminating in a burst of late gene expression that is regulated through a complex set of factors. We have previously shown that HSV1 lacking the virus protein VP22 (Δ22) exhibits late translational shutoff and fails to plaque on primary human fibroblasts. Intriguingly, Δ22 virus replicates and spreads as efficiently as Wt, but without causing cytopathic effect (CPE). Nonetheless, CPE-causing virus spontaneously appeared on Δ22-infected human fibroblasts, and four viruses isolated in this way had all acquired point mutations in the viral endoribonuclease, VHS, resulting in the rescue of late protein translation and CPE.

To determine the effect of these point mutations on VHS endoribonuclease activity outside of virus infection, we have expressed each of them ectopically alongside the Wt VHS protein. Unlike Wt VHS, all VHS variants were unable to relocalise the polyA binding protein PABP to the nucleus, or reduce expression of a reporter, indicating that they were not inducing RNA degradation. Furthermore, they induced large stress granules which we propose to be composed of translation machinery that has been stalled by the binding of inactive VHS. This data suggests that in the absence of VP22, there is a selective pressure on this virus to mutate VHS despite those mutations potentially inducing a blockade to translation. We are now using these degradation-defective variants to investigate the cellular binding partners of VHS and address why HSV1 is pressured to mutate VHS in this way in a VP22-negative background.

B229

Establishment of reverse genetics and fluorescent reporter viruses for influenza D virus

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Abstract

Influenza D virus (IDV) was first identified in 2011 in pigs in the United States and is now known to infect cattle, swine and small ruminants, with serological evidence of exposure in several other species. High seropositivity has been reported in humans, although no confirmed human infections have been documented to date. Previous studies demonstrated that IDV can efficiently replicate in human respiratory epithelial cells and utilise essential host factors, and recent studies have suggested an association between human IDV exposure and respiratory symptoms, raising the possibility of unrecognised, mild, or asymptomatic infections. The broad host range, widespread human exposure, and close similarity to the human influenza C virus all identify IDV as a potential pandemic risk, but tools for studying its fundamental virology are currently very limited. To facilitate mechanistic studies of IDV replication and host interactions, we developed two reverse genetics systems for IDV, one using the seven native genome segments and one, based on IDV's unusual assembly strategy of packaging an extra redundant genome segment into virions, using eight genome segments. We then adapted these systems to produce a reporter IDV that encodes the fluorophore ZsGreen. Those systems provide a versatile molecular platform for visualising viral replication, studying gene function, and advancing our understanding of IDV biology.

B230

Live cell confocal microscopy to visualize the replication dynamics of foot-and-mouth disease virus

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Abstract

Foot-and-mouth disease virus (FMDV) replication occurs at distinct sites within the cytoplasm of infected cells and is mediated by virally encoded non-structural proteins. One such protein is 3A, which can be used as a marker of replication sites. Here we have used reverse genetics to generate viable viruses carrying either a FLAG or HA epitope tag in the 3A replication protein.

To investigate replication dynamics by confocal microscopy, cells were co-infected at high MOI with two viruses encoding either FLAG-tagged or HA-tagged 3A, fixed and labelled using antibodies directed against the epitope tags. At early time points, puncta representing sites of replication within co-infected cells contained only HA-tagged 3A (red) or FLAG-tagged 3A (green) but not both, whereas at later time points 3A puncta were larger and contained both forms of 3A. These data indicated that early sites of replication were derived from individual incoming viruses, and that these early sites subsequently merged.

To investigate the dynamics of sites of replication more directly, we have developed systems whereby 3A protein from tagged viruses can be followed in real time in live cells. To this end, we have expressed engineered single chain anti-Flag and anti-HA antibodies tagged with GFP ("Frankenbodies") in cells, and subsequently infected those cells with virus carrying the FLAG or HA epitope tags in 3A. This approach allows the observation of the dynamics, transport and merging of sites of replication in real time.

B231

Exploring chikungunya virus's adaptability to a new vector species

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Abstract

Chikungunya virus (CHIKV), a single-stranded positive-sense RNA virus, causes recurrent outbreaks globally. A single mutation in E1 (A226V) enabled its transmission by *Aedes albopictus,* in addition to *Aedes aegypti*. This mutation has been shown to emerge as fast as 10 days post-infectious blood meal in *Aedes albopictus*, but not in *Aedes aegypti*, which highlights the virus's flexibility in adapting to a new vector. In contrast to other *Alphaviruses*, CHIKV transmission is limited to being vectored by *Aedes* mosquitoes. Building on this viewpoint, this project exploits CHIKV adaptation to an *Anopheles* cell line, which are normally non-permissive cells. Specifically, we aim to investigate the genetic adaptation that will enable CHIKV to replicate in *Anopheles* cell lines.

To probe this question, we are establishing a forced selection system by co-culturing susceptible *Aedes* and resistant *Anopheles* cells with a gradually increasing proportion of *Anopheles* cells and exposing those cells to CHIKV. This work will assess the limits of CHIKV genome plasticity and estimate the genetic barriers to cross-species replication. Whether CHIKV exhibits adaptive potential or strict host constraints, the findings will provide valuable insights for understanding viral emergence dynamics and improving arbovirus-outbreak preparedness for potential vector expansion.

B232

Probing the Tertiary RNA Architecture of Rotavirus A to Uncover Determinants of Viral Replication

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Abstract

RNA tertiary structures play essential roles in the replication of many RNA viruses. Traditionally, identifying specific higher-order RNA motifs that influence viral replication has required extensive mutational screening followed by structural validation using X-ray crystallography or cryo-EM. Here, we apply TMO-MaP, a recently developed chemical probing technique, to investigate putative tertiary RNA motifs in species A rotaviruses. Adenosines exhibiting enhanced reactivity to TMO relative to DMS, termed T sites, serve as markers of a distinct tertiary RNA motif.

We performed TMO-MaP and DMS-MaP *in vitro* on full-length RNA genomes from two distinct strains of species A rotavirus, enabling the generation of a high-confidence T-site reactivity profile. To validate these findings, targeted mutations were introduced near identified T-sites, and their effects on TMO and DMS reactivity were assessed *in vitro*.

We are currently employing reverse genetics to rescue recombinant rotaviruses bearing T-site disruptions to assess the phenotypic consequences of T-site mutations on viral replication.

B233

Using single molecule method to visulise and analyse the host- virus interaction

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Abstract

SARS-CoV-2 causes severe respiratory clinical symptoms and high mortality worldwide, leading to the COVID-19 pandemic. SARS-CoV-2 infection is associated with a dysregulated innate immune response, characterised by low IFN production and the occurrence of high cytokine and chemokine levels. Notably, SARS-CoV-2 RNA was detected in secondary tissues and replicate in organs, in some cases leading to the serious long-term complex syndrome of Long Covid. Transcriptomic studies have revealed that host transcriptional changes differ between the early and late stages of SARS-CoV-2 infection. Furthermore, single-molecule fluorescence in situ hybridisation (smFISH) enables visualisation of viral RNA distribution and quantification of RNA expression levels within individual cells, exploring a surprising variability abundant of SARS-CoV-2 RNA in infected cells. However, the mechanism that enable viral dissemination to secondary organ remain poorly understanding. In this study, we are investigating how SARS-CoV-2 spreads to the secondary tissue after infecting the respiratory tract, potentially contributing to long-term Covid. Here, we used smFISH to assess the impact of the Interferon-stimulated genes transcription levels for vRNA replication during infection. We detected that the MX1 mRNA were rarely detected in infected cells, while the high level of MX1 mRNA was detected in neighbouring cell. In conclusion, we data suggest that the transcription level of ISGs may affect viral tropism in cells.

B234

Molecular characterisation of the 2023/2024 reassortant of Oropouche virus

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Abstract

Oropouche virus (OROV) is an Orthobunyavirus transmitted primarily by Culicoides biting midges in Central and South America. Human infection typically causes an acute febrile illness characterised by severe headache, myalgia, and arthralgia, but recent outbreaks have included cases with neurological and haemorrhagic manifestations. During 2023–2024, OROV incidence markedly increased across the Americas, with the first fatal infections reported. The molecular basis for this apparent rise in pathogenicity and transmission remains unclear.

Here, we investigated molecular and vector-associated determinants of OROV infection by comparing a historical strain (BeAn19991) with two reassortant isolates (BC89/90) associated with recent outbreaks. The interferon antagonistic activity of each strain's non-structural protein (NSs) was assessed using transient reporter assays in mammalian cells, showing strong suppression of the RIG-I pathway. To examine replication dynamics and vector competence, Culicoides sonorensis (American) and Culicoides nubeculosus (European) cell lines were infected with each OROV strain, showing replication in the American midge KC cell line, but not in the European midge CNE/LULS44 cell line. Ongoing optimisation of transfection protocols aims to enhance expression studies in midge cells, while parallel efforts are using a reverse-genetics system to generate recombinant reporter viruses for live-cell imaging and mechanistic analyses.

Together, these studies provide new insights into the molecular and cellular features underpinning OROV immune evasion and vector adaptation. Our findings lay the groundwork for defining how reassortment and host–vector interactions may influence OROV emergence, transmission potential, and pathogenicity, contributing to improved understanding of Orthobunyavirus evolution and vector-borne disease risk in the Americas.

B235

Exploring Viral Interactions with Gastrointestinal Nematodes in a Swine Model System

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Abstract

The swine industry is the fastest-growing livestock subsector globally, but its rapid expansion raises concerns about the impact of pathogens, including helminths (such as the gastrointestinal nematode, *Ascaris suum*) and viruses (like influenza A virus, IAV), on pig health, productivity, and food security. Increasing anthelmintic drug resistance and limited vaccines further complicate control efforts, posing challenges for sustainable agriculture and One Health objectives. On often over-looked phenomenon is the co-occurrence of nematodes and viruses within a single host, which include viruses of pigs but also viruses of nematodes themselves. There is a limited understanding of the interactions between nematodes and these viruses at a molecular level. To this end, we utilise computational and *in vitro* models of the nematode-virus (*A.suum*/IAV) interface to explore the diversity and co-evolution of GINs and viruses, as well as the impact of GINs on virus infection and replication, with particular emphasis on identification and characterisation of antiviral effectors. We hope to provide new insights into host-pathogen interactions that may inform the development of novel therapeutics and integrated disease management strategies for livestock systems.

Session : Virus Forum: Pathogenesis

B239

Morphological variants of the influenza A virus differentially influence replication dynamics and resistance to neutralisation.

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Abstract

Influenza A viruses display morphological heterogeneity, existing as both spherical and filamentous particles. While viral morphology has long been observed, its role in pathogenesis, replication efficiency, and immune evasion remains underexplored. Using plasmid-based reverse genetics, we generated a toolbox of fluorescently tagged influenza A viruses with site-specific M1 mutations or M-gene swaps to induce spherical or filamentous phenotypes. Viral replication and morphology were assessed through single- and multi-cycle growth curves, plaque assays, and microscopy. Neutralisation, exit, and entry inhibitor assays (using anti-HA antibodies, neuraminidase inhibitors, macropinocytosis inhibitors, and endocytosis inhibitors) were performed to quantify morphology-specific viral resistance. Inducing a filamentous morphology in a normally spherical virus background resulted in a higher replication efficiency in the absence of any inhibitors in both single- and multi-cycle growth curves. We also observed that filamentous viruses were more resistant than spherical viruses to endocytosis inhibitors and neutralisation by anti-HA antibodies; however, both spherical and filamentous viruses were inhibited by a neuraminidase inhibitor. In contrast, the addition of a macropinocytosis inhibitor only neutralised filamentous viruses. Fluorescence microscopy was used to gain insights into the effects of viral morphology on cell-to-cell spread. We have demonstrated that viral morphology has a significant impact on influenza replication kinetics. These findings highlight morphology as an underappreciated determinant of influenza virus fitness.

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Peste des petits ruminants virus (PPRV) encoded non-structural protein V modulates p73 stability and apoptotic signaling

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Abstract

Background: *Peste des petits ruminants virus*, also known as *Morbillivirus caprinae* causes a deadly disease in sheep and goats resulting in severe financial losses due to significantly high morbidity and mortality rates. Viruses subvert host immune responses to promote their survival. PPRV-V also manipulates host antiviral strategies, however, its immunomodulatory effects on apoptosis remain unexplored.

Methodology: To identify the host cellular interacting partners of PPRV-V protein, proteomics approach coupled with LC-MS analysis was performed. To validate the interaction of PPRV-V and cellular p73, the pull-down assays and co-immunoprecipitation assays were performed. Additionally, confocal microcopy was performed to analyze the subcellular localization of the proteins. Further, the stability assay was used to test the effect of PPRV-V on p73 through Cycloheximide chase assay and ubiquitination assay. Moreover, the effect on apoptosis was monitored by investigating the modulation of cellular apoptotic proteins such as PARP.

Results: Our study revealed a list of probable interacting partners of PPRV-V. It also validated that PPRV-V directly interacts with p73 protein and accelerates its degradation via enhanced ubiquitination. Immunofluorescence analysis showed PPRV-V in both nuclear and cytoplasmic compartments whereas p73 was nuclear when both were over-expressed. However, PPRV-V expressed alone was found to localize in cytoplasm only. Also, PPRV-V was able to reduce PARP cleavage, an apoptosis marker, in a dose-dependent manner.

Conclusion: This study highlights the antagonistic role of PPRV-V in the modulation of p73-mediated apoptotic signaling, thus, providing a critical insight into the possible mechanism of programmed cell death evasion by morbilliviruses.

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Epstein Barr Virus (EBV) upregulates Human Endogenous Retroviruses (HERVs) in Monocytes and Microglia via Toll-Like Receptors (TLRs)

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Abstract

Multiple sclerosis (MS) is a disabling, progressive inflammatory disease of the central nervous system. MS is considered a multifactorial disease in which environmental triggers act on the background of genetic susceptibility. The greatest risk factor for developing MS is prior infection by Epstein-Barr Virus (EBV). The extent to which EBV acts as a trigger for developing MS or acts as a driver of MS progression, or both, remains unknown. However, there is increasing interest in the contribution of human endogenous retroviruses (HERVs) in the pathogenesis of MS. Previous reports suggest EBV infection can transactivate HERVs, triggering neuronal degeneration through expression of immunogenic genes including envelope (*env*), resulting in inflammation and neuronal damage.

We aimed to address this question by infecting B cells with purified EBV, examining how EBV transactivated the HERVs and understanding the downstream consequences. However, we found that neither EBV, B cell activation by CD40 ligation or EBV lytic replication in plasma cells transactivated HERVs in B cells. Instead, we found EBV specifically and significantly transactivated HERV-W and HERV-K in monocytes and microglia. Transactivation was mediated by stimulation of the Toll-like receptors (TLR) TLR3 and TLR9, likely via the EBV small non-coding RNAs (EBERs) and/or CpG unmethylated EBV DNA. We are currently confirming how EBV, activated TLRs and HERV transactivation alter the transcriptome and function of the microglia and will subsequently examine the consequences for myelination in a multicellular model.

B243

Investigating the immunological and functional importance of a novel antigenic domain (AD-6) identified in HCMV gB/MF59 vaccine studies

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Abstract

A vaccine against human cytomegalovirus (HCMV) remains an important priority. In ongoing investigations of the humoral immune response directed against the gB/MF59 vaccine candidate we recently identified a response against a novel vaccine-specific antigenic domain (AD-6) which we hypothesise is an important mechanistic correlate of protection. Specifically, anti-AD-6 antibodies potently inhibit reactivation and cell-associated spread of the virus. Our current focus is to combine structural, mechanistic and immunological approaches to study AD-6 in detail to better understand it's role in the function of gB.

A lack of sequence similarity across human herpes virus gBs, led us to take a structural approach to identify putative co-ordinates of AD-6 regions in other gBs. Using ELISA we showed that a rabbit HCMV AD-6 polyclonal antibody recognises and binds HSV-1 and EBV AD-6. Furthermore, human anti-AD-6 responses generated by gB/MF59 vaccinees similarly recognised HSV-1 AD-6 observing that binding to HCMV and HSV AD-6 was positively correlated suggesting that responses against conformational epitopes are an important component of the gB/MF59 vaccine response.

To better understand the polyclonal AD-6 response, we divided the peptide into 3 fragments (A-C) based on structure. and observe that although antibody responses to AD-6 fragments were lower than the full-length AD-6 peptide responses against fragments A and C were highest, with fragment B staying at baseline. Together these data build our understanding of the anti-AD-6 antibody response and argue for antibodies directed against conformational epitopes in gB/MF59 vaccine recipients could be important for the protective response seen in vaccinated transplant patients.

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Genetic Susceptibility to Severe Pediatric Influenza Infection

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Abstract

Background. Influenza remains a major cause of pediatric morbidity and mortality worldwide. Several factors can increase the risk of severe disease. *IFITM3* protein is a critical antiviral factor restricting influenza virus infection. The single nucleotide polymorphism (SNP) rs34481144 (C/T) in *IFITM3* has been associated with impaired immune responses. We aimed to determine whether the rs34481144 variant is associated with influenza infection severity in children.

Methods. Prospective cohort study including children <16yo hospitalized with acute respiratory infection during 2019-2023 at the Ricardo Gutierrez Children´s Hospital. Patients who did not require supplemental oxygen therapy (SO_2) were defined to have mild disease; severe cases were those who required SO_2. Nasal aspirates were tested by RT-qPCR for influenza A/B. DNA was isolated from samples for genotyping and *IFITM3* mutations were assessed by allelic discrimination with pre-optimized assays and performed by RT-qPCR.

Results. 401 children were enrolled. 51 (13%) were positive for influenza. Of them, 27 (53%) were male, the median age was 12 months. All were infected with influenza A type. Thirty-nine (76%) had severe disease. To determine whether SNP rs344811 was associated with Influenza disease severity, genotype was compared between groups. The need of SO_2 was increased among carriers of the alternative allele, with a significant detrimental effect p=0.0069. Furthermore, patients carrying the alternative allele had significantly lower oxygen saturation on admission (p=<0.001). No significant differences were observed in viral load or sex distribution across genotypes.

Conclusions. The *IFITM3* rs34481144-T allele is associated with increased severity of influenza in children.

Session : Virus Forum: Transmission, Epidemiology & Diagnostics

B250

Integrated Indexing Multi-Segment (iiMS) Library Preparation and Viral Nucleic Acid Extraction Methods for Influenza A

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Abstract

This poster presents two areas of method development conducted by scientists at New England Biolabs. The first area focuses on a robust workflow for library preparation and sequencing of Influenza A, designed to support public health monitoring of respiratory disease outbreaks through whole genome sequencing. The targeted cDNA amplification approach streamlines reverse transcription and amplification into a single step using a high-fidelity enzyme mix, which reduces plastic consumption and minimizes sample cross-contamination. The integrated indexing multi-segment (iiMS) method incorporates Oxford Nanopore Technologies (ONT) Native Barcode sequences within the indexing primers, enabling efficient demultiplexing of 4–96 samples using standard ONT settings. This protocol achieves high, balanced coverage across all eight gene segments, producing high-quality data for research and public health applications with reduced time and material costs.

The second area addresses the challenges of viral nucleic acid isolation, emphasising the need for high sensitivity and throughput to meet the demands of viral surveillance. The Monarch® Mag Viral DNA/RNA Extraction Kit was developed for hands-free operation on automated platforms, offering flexibility and versatility. While saliva and respiratory swabs in Viral Transport Media (VTM) are common sample types, the USDA recommends using milk and nasal swabs for H5N1 testing in cows. The extraction method was evaluated for milk and VTM following reports in 2024 of HPAI A (H5N1) outbreaks in dairy cows. RT-qPCR results demonstrate that the kit enables successful extraction of viral nucleic acids from both VTM and milk, with minimal differences in performance.

B251

Evaluation of five commercial methods for the co-extraction of DNA and RNA from a panel of inhibitory matrices ahead of downstream nucleic acid analysis

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Abstract

Molecular methods - including PCR and metagenomic sequencing - depend on efficient and effective nucleic acid extraction to generate high quality, purified RNA or DNA for analysis. If there is limited information on the pathogen present within a sample, optimised methods for the co-extraction of RNA and DNA are critical ahead of the analysis techniques used for the identification of the infection.

Here we evaluated whether a single nucleic acid extraction kit could be used to effectively and robustly to generate high quality, purified RNA and DNA. Five commercially available kits were assessed for the extraction of both bacterial DNA and viral RNA simulant from a range of spiked matrices (e.g. horse blood, baking powder, soil). The efficiency of each extraction was measured by PCR. To evaluate whether there was any evidence of inhibitory molecules being carried over by each kit, the final extracts were diluted and the performance of the PCR compared with neat samples.

The QIAamp Viral RNA mini kit broadly performed best across all metrics used to evaluate performance (i.e. limit of detection and sensitivity). On a practical level, the QIAamp Viral RNA mini kit also has the added advantages of a short run time, requires minimal equipment and lower operator burden when compared with other spin-column-based methods. Overall, the QIAamp Viral RNA mini kit was found to perform strongly for DNA and RNA extraction irrespective of the matrices used and therefore is recommended for use when the nature of the pathogen remains unknown within a sample.

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B252

Monitoring respiratory syncytial virus genomic diversity in the United Kingdom using Addenbrooke's Hospital in Cambridge as a sentinel site

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Abstract

Respiratory syncytial virus (RSV) is a major cause of acute lower respiratory tract infections in children, causing global morbidity and significant demand on healthcare systems. In the United Kingdom, monoclonal antibody treatments and vaccines are available, with both strategies targeting the F protein to immunise against both RSV subtypes (A and B). In this context, genomic surveillance of RSV is necessary to track mutations that could lead to immunological escape and influence the efficacy of the immunisation programme. In this study, we examine the effectiveness of using Cambridge, UK, as a sentinel site to detect the diversity of circulating RSV strains in the UK. Patients with symptoms of respiratory infection at Addenbrooke's Hospital were initially screened using qPCR. Libraries from positive samples were prepared using a hybrid capture technique and sequenced using an Illumina platform. Consensus sequences revealed 26 RSV-A and 18 RSV-B clades circulating in the UK between 2023 and 2025. Of these, eight RSV-A and five RSV-B clades were undetected in Cambridge. Nevertheless, there was a high positive correlation between Cambridge and the rest of the UK for observed mutations, particularly for those present in the F protein. Phylodynamic analysis revealed that RSV A and B sublineages were introduced with a small time lag between Cambridge and other UK regions. This suggests that a single hospital could represent a broader RSV viral population circulating in the UK.

B254

Investigation into the role of biting flies in the mechanical transmission of African swine fever virus.

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Abstract

Investigation into the role of biting flies in the mechanical transmission of African swine fever virus.

African swine fever (ASF) is a devastating, often fatal, haemorrhagic disease of domestic swine and wild boar caused by the African swine fever virus (ASFV). The lack of a widely approved and effective vaccine makes ASF control particularly challenging.

Effective control relies on understanding its transmission epidemiology, which includes biological transmission by soft argasid ticks in Africa, and direct/indirect spread (e.g. via contaminated pork products or human activity). Previous work has shown potential involvement of arthropods as mechanical vectors in ASFV transmission, although there is limited supporting data. The stable fly, *Stomoxys calcitrans*, has been highlighted as a potential mechanical vector, and other blood-feeding arthropods such as mosquitoes may also have capacity for ASFV transmission.

We have shown that viral DNA remained detectable in stable fly bodies up to four days after feeding on ASFV-infected pigs. Low levels of viral DNA were recovered from isolated fly head samples post-feeding, and infectious ASFV was found within whole fly samples up to two days post-feeding. In ongoing work, we are investigating ASFV transmission by stable flies to naïve pigs and determining biting rate through the detection of pig DNA in the fly samples by qPCR. We will also assess ASFV acquisition and stability in stable flies, mosquitoes and biting midges. This work will evidence the potential role of mechanical transmission of ASFV to support epidemiological modelling and inform the advice on preventing ASFV spread.

B255

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

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Abstract

While airborne transmission of viruses has long been a significant area of research, foot-and-mouth disease virus (FMDV) is relatively understudied in this regard. This is an important gap as FMD has severe economic impacts; the 2001 outbreak cost the UK approximately £8 billion, in addition to the culling of 6.5 million animals. Although the primary mechanism of transmission for FMDV is direct contact between cloven-hooved animals, a low probability but high consequence transmission mechanism is via aerosols. Aerosols can travel much further distances than larger droplets and therefore have the potential to circumvent quarantine zones, an important part of FMD control. This, combined with the fact that several outbreaks of FMD have likely resulted from aerosol transmission, demonstrates the importance of studying the environmental conditions determining if infectivity is retained during aerosol transport.

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

B256

Nanopore Sequencing with R9.4 Flow Cells Reliably Detects HIV Drug Resistance Mutations.

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Abstract

Background:
Monitoring HIV-1 drug resistance (HIVDR) is critical for maintaining antiretroviral therapy (ART) effectiveness. Sanger sequencing is the gold standard for detecting *pol* gene resistance mutations, but low throughput and labour-intensive workflows limit large-scale use. Oxford Nanopore sequencing offers rapid, high-throughput, long-read sequencing with multiplexing. We aimed to benchmark a Nanopore-based workflow for HIVDR detection across protease (PR), reverse transcriptase (RT), and integrase (IN), comparing results with Sanger sequencing.

Methods:
Paired plasma samples (n = 119) were used for Sanger and Nanopore sequencing. Viral RNA was extracted, reverse-transcribed to cDNA, and PR/RT and IN fragments amplified by nested PCR. Nanopore libraries were prepared with 96-plex barcoding and sequenced on MinION for 72 hours using R10.4 flow cells. Resistance profiles were determined using the Stanford HIV Drug Resistance Database (HIVdb). Data are unfiltered for quality.

Results:
PR/RT concordance ranged 0–100%, with 61% fully concordant and 22% >75% concordant. IN concordance ranged 0–100%, with 58% fully concordant and 21% >75%. Overall codon-level concordance was 52% for PR/RT and 55% for IN. Discordances mainly reflected differences in codon-frequency thresholds rather than sequencing errors. Subgroup analysis showed PR highest (68% full concordance), RT intermediate (59%), IN lowest (58%). Full gene coverage was achieved for all samples, and quality filtering and sequence polishing are expected to improve agreement.

Conclusion:
Nanopore sequencing enables rapid, high-throughput HIVDR genotyping. With optimised quality filtering, it is a viable alternative to Sanger sequencing for clinical and public health applications.

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