ASIA-PACIFIC BIOFILMS CONFERENCE

2026

HOSTED BY: CENTRE TO IMPACT AMR

ABSTRACT BOOK

MARCH 27TH - 29TH

Lecture Theatre,
Innovation and Education Hub
THE ALFRED HOSPITAL
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WELCOME MESSAGE

It is our great pleasure to welcome you to the Asia-Pacific Biofilms Conference. This meeting brings together researchers, clinicians, industry partners, and students from across the Asia-Pacific region and beyond to share recent advances in the study of microbial biofilms and to discuss emerging challenges and opportunities in the field.

The 2026 conference program includes plenary talks, invited talks, selected presentations, and a poster session that reflect the breadth of biofilm research across the region. Topics span fundamental biofilm biology, microbial ecology, host–microbe interactions, antimicrobial strategies, medical biofilms, environmental and industrial biofilms, and emerging technologies for studying complex microbial communities.

The conference also aims to support the next generation of scientists. We are particularly pleased to welcome students and early-career researchers, whose ideas and contributions will help shape the future of biofilm science.

We hope this meeting provides an engaging forum for discussion, inspiration and collaboration. We thank all speakers, presenters, and participants for their contributions to the conference.

The Asia-Pacific Biofilms 2026 Organising Committee

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ABOUT THE ORGANISERS

Main organiser

The Centre to Impact AMR (Monash University) focuses on delivering research-driven solutions to the global challenge of antimicrobial resistance. Its work spans microbial biology, infection prevention, antimicrobial discovery, diagnostics, and stewardship strategies. The Centre aims to translate research into real-world impact, inform clinical practice and policy, and support the development of emerging researchers. Through national and international collaboration, it contributes to coordinated efforts to reduce the burden of antimicrobial resistance.

Monash Centre to Impact AMR

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The Mark Shirtliff Biofilm Foundation works to advance biofilm research and community engagement worldwide. Its activities include mentoring and educational support for early-career researchers, fostering scientific exchange, and raising funds to support research visits, conference participation, and related opportunities. The Foundation seeks to broaden the reach and impact of biofilm science by promoting the development of future research leaders. Those wishing to support the Foundation in its mission can learn more and make donations via the website: markshirtliffbiofilmfoundation.org.

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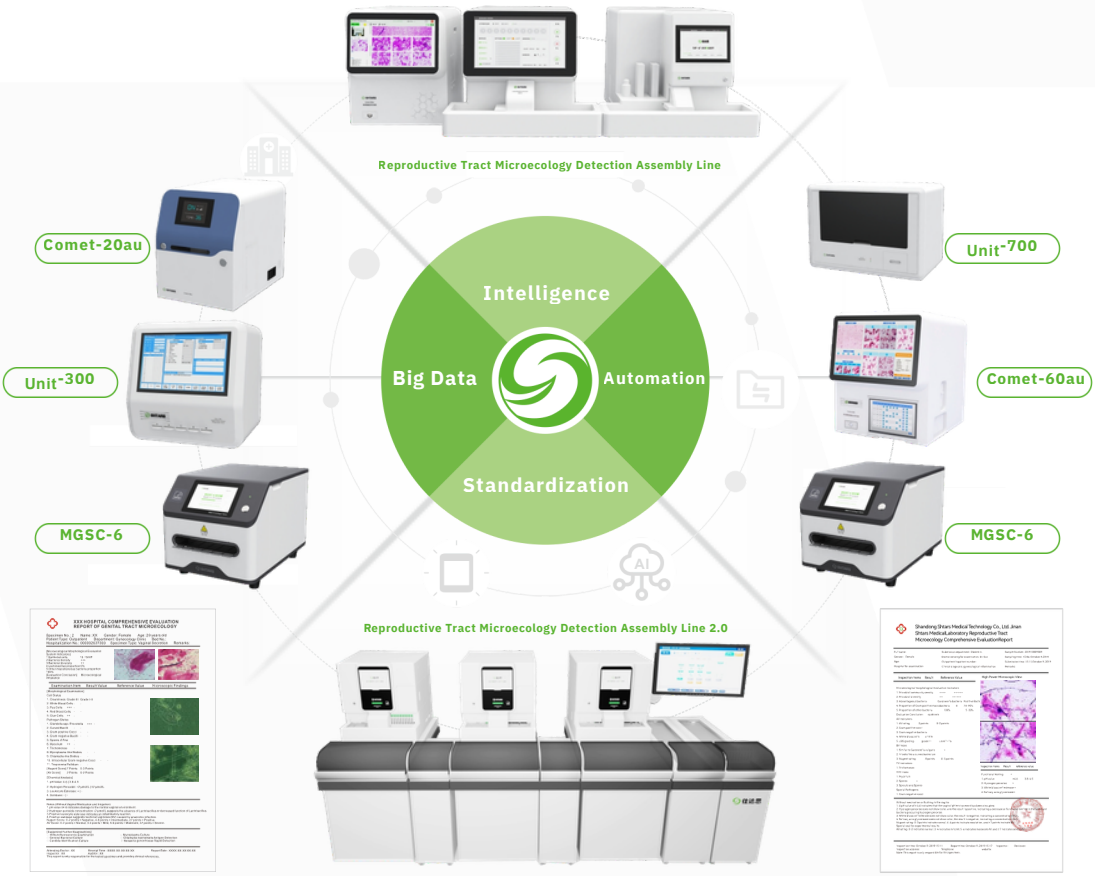

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PLENARY ONE

Professor Gordon Ramage

Glasgow Caledonian University, United Kingdom

The Clinical Impact of Fungal and Interkingdom Biofilms

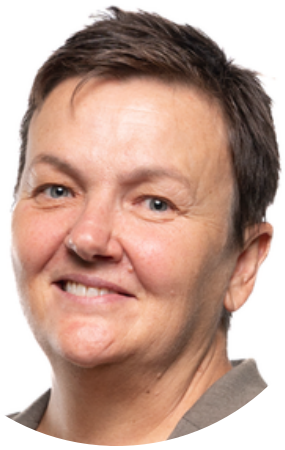
Biofilms are structured microbial communities embedded within an extracellular matrix that confer protection against environmental stress and antimicrobial therapies. While bacterial biofilms have historically received considerable attention, fungal biofilms—particularly those formed by *Candida* species—are increasingly recognised as important contributors to chronic infection. In many clinical settings, these fungal communities exist alongside bacteria, forming interkingdom biofilms that exhibit complex ecological interactions capable of influencing virulence, persistence, and treatment outcomes.

This presentation will examine the clinical significance of fungal and bacterial–fungal biofilms in two key infection contexts: the oral cavity and chronic wounds. In the oral environment, interkingdom biofilms involving *Candida albicans* and oral bacteria such as streptococci contribute to conditions including denture stomatitis and oral candidosis, where synergistic interactions enhance biofilm biomass, structural stability, and tolerance to antifungal agents. Similarly, in chronic wounds, polymicrobial biofilms frequently contain fungal organisms alongside bacterial pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, resulting in communities that are highly resilient to host immunity and conventional antimicrobial therapies.

Using examples from experimental studies, the presentation will highlight the development of clinically relevant biofilm models that capture aspects of microbial complexity, surface attachment, and host-relevant conditions. These models include *in vitro* and *ex vivo* systems designed to mimic oral surfaces and wound environments, enabling mechanistic studies of microbial interaction, biofilm architecture, and antimicrobial tolerance. Such models provide essential platforms for evaluating novel antimicrobial strategies, including antifungal agents, antibiofilm compounds, and surface-directed interventions.

Together, these studies illustrate how interkingdom microbial interactions can drive pathogenicity and therapeutic resistance, underscoring the need for experimental systems that reflect the polymicrobial nature of many infections. The presentation will conclude by discussing how advances in biofilm modelling can support the development of more effective antimicrobial interventions targeting complex microbial communities in oral and wound infections.

PLENARY TWO



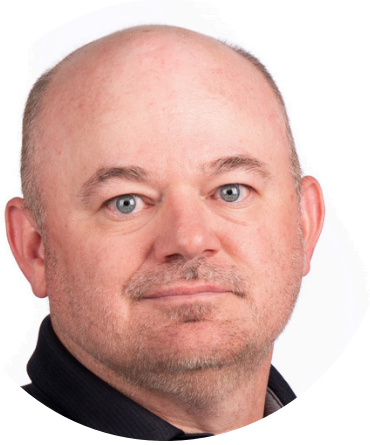
Professor Cynthia B. Whitchurch

Singapore Centre for Environmental Life Sciences Engineering (SCELSE), Nanyang Technological University, Singapore, Singapore

When Death Becomes a Biofilm: Autolytic Programmed Cell Death in *Pseudomonas aeruginosa* Biofilms

Programmed cell death (PCD) - genetically regulated cell suicide - has long been considered a hall-mark of multi-cellularity in eukaryotes as it is essential for organismal development and homeostasis by removing unnecessary or damaged cells. Whist it seems counter-intuitive that single-celled bacteria would possess cell suicide pathways, it is now increasingly evident that PCD is beneficial in the context of multicellular bacterial communities. *Pseudomonas aeruginosa* undergoes autolytic PCD that culminates in explosive cell lysis events that release extracellular DNA (eDNA) and other cytosolic materials required for initiating and building biofilms. This process is regulated by the RecA- mediated SOS regulon and requires the endolysin, Lys, encoded in the R-/F- tailocin (pyocin) gene cluster. The R- and F- tailocins are bacteriocins that are evolutionarily related to phage tails and are released via a conserved phage lysis system comprised of the endolysin, Lys that degrades the cell wall peptidoglycan and a holin, Hol, that accumulates in the inner membrane (IM) until it is triggered to assemble as a pore to transport Lys from the cytoplasm to the periplasm. In Gram negative bacteria, the final step of phage lysis requires the activity of spanins that promote rupture of the outer-membrane (OM) by forming a complex that spans the IM and OM and fuses these membranes. We have determined that the gene products of PA0630 and PA0631, encoded downstream of *lys*, appear to be the spanin components of this PCD pathway. Autolytic PCD in *P. aeruginosa* during biofilm development and maturation also incorporates the AlpB-E and CidAB autolysis pathways. Therefore it appears that *P. aeruginosa* has co-opted and integrated three autolysis pathways into a programmed cell death program that regulates the production of tailocins, and eDNA and other public goods required for biofilm development.

PLENARY THREE



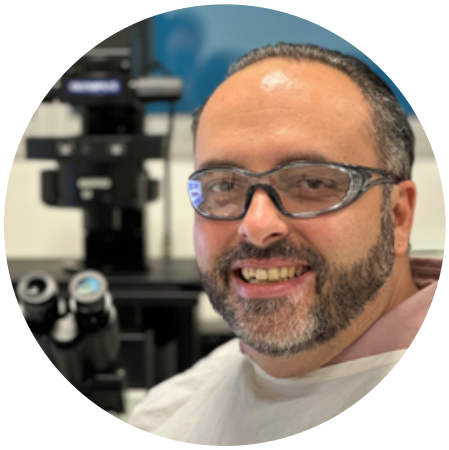
Professor Marvin Whiteley

Georgia Institute of Technology and Emory Medical School, USA

From Bedside to Bench: Using Human Infection Data to Guide Discovery

Bacterial behavior and physiology during human infection is difficult to study and largely unknown, as our vast knowledge of infection microbiology is primarily derived from studies using *in vitro* and animal models. A key challenge to assessing bacterial physiology during human infection is the difficulty in acquiring and assessing bacterial function in human-derived samples. Here, I will discuss the use of microbial metatranscriptomics from chronic human wound and lung infections to tackle this gap in knowledge. We have leveraged these data in two primary ways: to assess and improve the accuracy of preclinical biofilm infection models using a quantitative framework recently developed in our lab; and identifying and functionally characterizing genes of unknown function that are highly expressed in humans but not in most preclinical models. I will also discuss additional approaches we are using to quantify biofilm biogeography and heterogeneity within human infections, with the goal of using these data to develop accurate preclinical models and guide antimicrobial use.

INVITED SPEAKER ONE



Zlatko Kopecki

Adelaide University & PolyNovo Biomaterials Pty Ltd, Australia

Novel Approaches for Management of Biofilm Wound Infections

Smart materials that provide targeted antimicrobial delivery together with healing properties present a promising opportunity to address the increasing threat of bacterial biofilms and clinical infection. Here we describe two approaches being developed for management of bacterial biofilms: a stimuli responsive dressing for acute wound infections and an AMP coated biotemporising matrix for management of deep burn injuries. Safety and efficacy of a pH/temperature responsive silver nanoparticle hydrogel against industry standard of care (silver sulfadiazine) was demonstrated using a pre-clinical porcine model of acute wound infection. *In vivo* validation in *Staphylococcus aureus* infected excisional wounds demonstrate high efficacy at significantly lower doses of Ag+. Second approach describes development of a next-generation antimicrobial dermal substitute (BTM-AMPs) using Nisin and LL-37. *In vitro* results demonstrated strong antimicrobial activity of the BTM-AMPs, with an ability to eradicate mature polymicrobial biofilms consisting of common Gram-positive and Gram-negative pathogens. In a direct contact kill assay, BTM-AMPs showed a comparable efficacy to the commercially available antimicrobial matrix, Endoform™. Using an *ex vivo* bioluminescent biofilm model, BTM-AMPs reduced the burden of metabolically active bacteria by >50% after 6 hrs of treatment. Furthermore, BTM-AMPs exhibited strong anti-inflammatory properties by significantly reducing the level of pro-inflammatory TNF-α cytokine and increasing the phagocytotic activity of macrophages *in vitro*. Overall, both approaches present promising platforms for managing clinical infections and eradicating biofilms in acute and chronic wounds.

INVITED SPEAKER TWO

Tom Coenye

Ghent University, Belgium

Translating Biofilm Microenvironment Insights into Next-Generation Diagnostics and Susceptibility Testing for Biofilm Infections

Tom.Coenye@UGent.be

Biofilm-associated infections represent a major clinical challenge, largely due to the profound influence of the local microenvironment on microbial behavior, diagnostic detectability, and antimicrobial susceptibility. Although biofilms occur both as surface-attached communities and as suspended or tissue-associated aggregates, conventional diagnostic and susceptibility testing methods continue to rely predominantly on planktonic cultures grown in artificial laboratory media. As a result, these approaches frequently fail to replicate the physiological conditions present at infection sites, contributing to poor predictive value and suboptimal clinical outcomes.
In my talk I will explore how insights into biofilm microenvironments can be leveraged to design next-generation diagnostic tools and susceptibility assays. Data from osteoarticular and implant-associated infections demonstrate that many pathogens form robust aggregates in synovial fluid or synovial fluid–mimicking media. Using synthetic synovial fluid (SSF), my team has shown that microbial physiology, antimicrobial tolerance, and biofilm formation more closely resemble *in vivo* conditions than when grown in standard rich media. Our work has also revealed that minimal inhibitory concentrations (MICs) poorly predict biofilm-related parameters such as biofilm-preventing concentration (BPC) and minimal biofilm inhibitory concentration (MBIC), with substantial strain- and antibiotic-specific variation.
Beyond susceptibility testing, SSF markedly improves culture-based detection of prosthetic joint infection pathogens, increasing both positivity rates and detectable microbial diversity. Additionally, isothermal microcalorimetry enables rapid detection of microbial growth in under 24 hours, offering a promising tool for accelerating diagnosis.
Together, these findings illustrate that incorporating microenvironment-mimicking conditions into diagnostic pipelines can enhance detection, refine susceptibility testing, and potentially guide more effective treatment strategies for biofilm-related infections.

INVITED SPEAKER THREE

Kirby Lattwein

Erasmus University Rotterdam, Netherlands

Innovations in Biofilm-Driven Cardiovascular Infections: From Diagnosis to Treatment

Biofilm-driven infections within the cardiovascular are life-threatening and, despite technological advances over recent decades, remain a major clinical challenge. These infections range from those of native and prosthetic tissue inside the heart to device-associated infections involving life-sustaining technologies, such as pacemakers (which regulate heart rhythm) and ventricular assist devices (mechanical pumps that support heart function). Diagnosis is difficult, as symptoms are often non-specific, and clinical decision-making remains complex, from antibiotic selection and treatment duration to the timing of surgical intervention. Current approaches are often insufficient, resulting in delayed or suboptimal treatment and poor clinical outcomes.

Recent advances and innovations in the field are actively addressing these challenges, ranging from multidisciplinary team discussions for patient management to the development of databases that support clinical decision-making and research. Efforts in prevention, including improved early identification and monitoring, are complemented by innovations in device design and treatment strategies, ranging from optimized antibiotic protocols to alternative and adjunct antibiofilm therapies. These efforts are supported by ongoing research that aims to better understand biofilm behavior and translate these insights into clinical solutions, alongside the use of advanced imaging and patient-derived models that more accurately reflect real-life infections.

In this talk, I will highlight these advances and discuss how a more integrated, multidisciplinary approach can move the field toward prevention, earlier detection, improved monitoring, and targeted intervention, alongside promising (pre)clinical research. Altogether, getting to the heart of biofilm-driven cardiovascular infections.

INVITED SPEAKER FOUR

Birthe Kjellerup

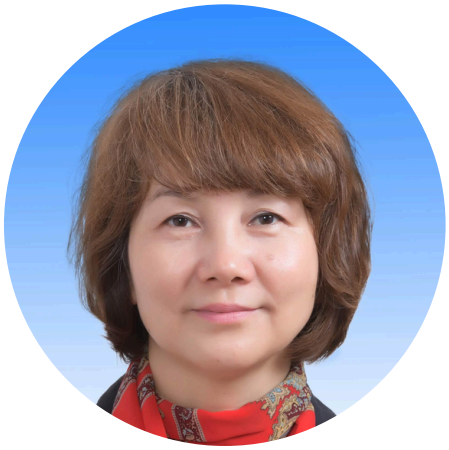
University of Maryland, USA

Biofilm and Anti-Fungal Resistance Characteristics of the Emergent Fungus *Candidozyma auris* (Previously *Candida auris*) in the Environment and Healthcare Settings

Yuzhu Mao[1], Mara Chen-Goldberg[1], Chunfu Liu[2], Rachel Hamant[1], Birthe Kjellerup[1]
[1] Department of Civil and Environmental Engineering, University of Maryland, College Park, College Park, MD, United States. [2] Montgomery County Department of Health and Human Services, Rockville, MD, United States.

Candida auris is an emerging multidrug-resistant fungal pathogen that represents a growing public health concern due to its persistence in healthcare environments, limited treatment options, and high transmissibility. Although closely related *Candida* species have been identified in diverse environmental habitats, the ecological niches and reservoirs of *C. auris* remain poorly understood. In particular, the environmental conditions that may facilitate its persistence, dissemination, and evolution of antifungal resistance are largely unknown. We hypothesize that wastewater and harsh engineered environments may promote genetic adaptations enhancing the survival and drug resistance of *C. auris*. To investigate this, a 12-month wastewater surveillance study was conducted across five regional sewer sheds in the USA. Twice weekly, 24-hour composite wastewater samples (n = 425) were collected from pumping stations. Samples were enriched in Sabouraud Salt Dulcitol (SSD) broth for up to seven days, and *C. auris* was isolated using selective agar. Presumptive colonies were confirmed by qPCR targeting the ITS2 region and identified by MALDI-TOF MS. Antifungal susceptibility testing was performed following CLSI M27-A4 guidelines, and biofilm formation capacity was quantified using crystal violet assays. *C. auris* DNA was detected in 19.7 % (84/425) of samples (Cq < 37), while viable isolates were recovered from 4.2 % (18/425). Positive detections occurred year-round across all sampling sites. Notably, *C. auris* remained viable in wastewater-inoculated SSD cultures stored at 4˚C for more than two years, indicating remarkable persistence. All isolates exhibited resistance to fluconazole (100%, 18/18) and a high prevalence of amphotericin B resistance (83.3 %, 15/18), substantially exceeding resistance levels typically reported for clinical strains (~30%). Additionally, four isolates demonstrated significantly greater biofilm formation capacity than CDC 0385, the reference strain used in EPA antimicrobial efficacy testing. This study provides information of viable *C. auris* in domestic wastewater, suggesting broader environmental presence than clinical surveillance alone indicates. Wastewater biofilms may represent a previously underrecognized ecological niche supporting survival and potentially facilitates the evolution of antifungal resistance in *C. auris*.

INVITED SPEAKER FIVE



Luyan Ma

Institute of Microbiology,
Chinese Academy of Sciences,
Beijing 100101,
China

Bacterial Self-Produced Enzymes that Inhibit Biofilm Formation and Enhance Biofilm Eradication

Luyan Ma*, Yu Zhang, Dejian Liu
* Luyanma27@im.ac.cn

Biofilm bacteria display extreme tolerance to almost all antibiotic classes and are protected from the host immune system, giving rise to chronic infections that are notoriously difficult to eradicate. Here we show bacterial self-encoded enzymes that target the bacterial cell wall or exopolysaccharide can inhibit biofilm formation and enhance biofilm eradication when supplied exogenously. Such enzymes can also enhance the activity of several antibiotics against *Pseudomonas aeruginosa*. Our results open a new path for antimicrobial discovery and anti-biofilm strategies.

INVITED SPEAKER SIX

Xiaoxue Wang

University of Chinese Academy of Sciences, Beijing[1] and South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou[2], China

Prophage-Encoded Toxin-Antitoxin Systems Mediates Biofilm Formation and Phage-Host Symbiosis

Xiaoxue Wang[1,2]*, Yunxue Guo[1,2], Jiayu Gu[1,2], Ran Chen[1], Shituan Lin[1,2], Kaihao Tang[1,2]

Since their discovery in conjugative plasmids in the 1980s, toxin-antitoxin (TA) systems have been found to be widely distributed across microbial genomes and horizontal genetic elements. To date, eight distinct types of TA systems with different molecular mechanisms have been identified, and they are involved in multiple dimensions of microbial life.

Filamentous Pf bacteriophages are widely distributed in *Pseudomonas aeruginosa* and profoundly influence biofilm formation and virulence. In recent studies on the activation of prophage Pf during the biofilm formation of *P. aeruginosa*, we found that a novel phosphorylation-based KKP TA system encoded within its integrated Pf6 prophage dynamically regulates Pf activation during biofilm formation through a reversible post-translational modification on the host silencer MvaU protein. This KKP TA system consists of a toxin composed of two tandem Ser/Thr kinases and a phosphatase antitoxin. Phosphorylation of MvaU by the two kinases represses another integrated Pf4 prophage excision and activation (Li et al., Mol Microbiol 2019; Guo et al., 2024 Nat Commun). Additionally, the antitoxin PfiA encoded by Pf4 is also the target of the two Ser/Thr kinases, and we revealed that the phosphorylation of the PfiA shifts the PfiAT complex stoichiometry from a noncanonical $PfiA_6PfiT_4$ to a canonical $PfiA_2PfiT_2$ assembly. This post-translational modification eliminates the pool of free PfiT toxins through complex reorganization, a process that permits Pf4 activation (Chen et al., Sci Adv, under revision). KKP also monitors the Pf4 activation by interacting with the replication-related protein RepG of Pf4 (Gu et al., 2025 Nat Commun). Thus, the KKP TA along with the PfiTA TA, act as the molecular switch governing biofilm formation and phage-host symbiosis inside the biofilm community.

INVITED SPEAKER SEVEN

Thomas W. Seviour

Aarhus University,
Denmark

Biofilm Solutions Through an Understanding of the Extracellular Polymeric Substances

The extracellular polymeric substances (EPS) are the essence of biofilms, yet their identities are poorly understood. There are few common EPS that behave similarly in different biofilms, and no universal biofilm EPS described. What we know about EPS originates from studies on laboratory systems. Attempts to transfer this knowledge to complex biofilms that predominate in environmental and clinical settings, often fail, and descriptions of EPS in complex systems are imprecise. Therefore, despite their importance, the EPS are inadequate targets for broad spectrum, specific interventions, to prevent or optimize biofilm formation. I will present here efforts to address this shortcoming in clinical and environmental systems.

Extracellular DNA is an EPS observed across multiple biofilms, including some complex biofilms. By studying eDNA in *Pseudomonas aeruginosa* biofilms we resolved how higher order structural motifs determine the ability of DNA to transition to viscoelastic extracellular matrix structures. Specifically, these included the observations of G-quadruplex, extracellular RNA, and finally extracellular R-loops, as critical. We then identified that SOS protein recA implants toxic R-loops into the genome of suicidal cells, under the regulation of the stringent stress response (relA), to promote DNA release. We could thus identify EPS targets for *P. aeruginosa* biofilm control.

In contrast to *P. aeruginosa*, activated sludge however represents a complex and intractable biofilm for EPS study. It is nonetheless arguably the most important biotechnological biofilm worldwide. Different approaches are required to study sludge, and I will present here the isolation and identification of a common and highly abundant sludge biopolymer. The presence of this biopolymer suggests a potentially common pathway for matrix production, for sludge and other biofilms, that was previously not considered.

With improvements in the resolution of EPS characterization for pure and complex biofilms, the understanding of EPS from both will begin to converge, hopefully enabling universal management strategies.

INVITED SPEAKER EIGHT

Jillian Cornish

University of Auckland,
New Zealand

Lactoferrin Enhances Biofilm Eradication in Bone Infections

Biofilm formation is a key contributor to recalcitrance of antibiotics in bone infections predominantly due to antibody tolerance. Our hypothesis is that we can enhance antibiotic activity with a second agent, sometimes termed an “antibiotic adjuvant”. We have identified bovine lactoferrin (bLF), an 80-kDa iron-binding milk glycoprotein, as a promising antibiotic adjuvant with anti-biofilm properties, leading to more effective eradication of bacterial infections. We have identified bLf as a factor that enhances antibiotic killing of *Staphylococcus aureus*. The feature of biofilm infection is tolerance to antibiotics due to the survival of a subpopulation of biofilm bacteria, where laboratory tests on planktonic cells indicate susceptibility. Tolerance is seen in bone infections of osteomyelitis and prosthetic joints, where methicillin-susceptible *S. aureus* (MSSA) strains predominate, but where treatments with the frontline penicillinase-resistant antibiotic flucloxacillin (FLU) can be ineffective. We have grown biofilms of MSSA, in a CDC Biofilm Reactor, then treated them with a combination of FLU and bLF and this significantly enhanced biofilm eradication efficacy. Co-administration of bLF with FLU resulted in a >6-log reduction in media CFU within two days, and mature biofilms on the metal coupon surfaces were almost completely eradicated after 3 days. We then developed a biodegradable hydrogel-niosome system to achieve sustained drug release from a single application. Biofilm eradication of FLU and LF under this *in vitro* local delivery system enhanced biofilm killing ($P < 0.05$), providing an effective approach for delivering LF and FLU to biofilms *in vivo*. We are now doing *in vivo* studies in a rat bone infection model, using a hydrogel–niosome system that enables controlled co-release of FLU and LF to eradicate biofilms and this is demonstrating the system's potential for localized biofilm clearance *in vivo* in a safe and effective manner.

Bone infection can impede blood circulation within the bone, leading to bone death. LF as a potent stimulator of bone growth adds to its appeal as a safe and effective treatment for bone infections.

INVITED SPEAKER NINE

Jintao Liu

Tsinghua University, School of Basic Medical Sciences, China

Spatial Heterogeneity and Resistance of Biofilms

JintaoLiu@tsinghua.edu.cn

A key feature of biofilms is spatial heterogeneity. Due to the dense packing of the bacteria, spatial gradients emerge within the community. It remains unclear how combinations of different gradients shape biofilm properties. By modulating the penetration of nutrient and oxygen, we demonstrated that combination of nutrient and oxygen gradients gives rise to two types of biofilms with divergent modes of metabolism. We further found that one type of biofilm displayed significantly higher tolerance to antibiotics than the other. This enhanced tolerance is attributed to low membrane potential, which reduces antibiotic uptake. Supplementation of metabolites that enhance respiration markedly potentiated antibiotic-mediated killing of the biofilm. Our work reveals how combination of gradients gives rise to different types of biofilms, advancing our understanding of their stress response, facilitating the development of more effective eradication strategies.

Biography: Jintao Liu, Associate Professor at Tsinghua University, School of Basic Medical Science. BS in physics from University of Science and Technology of China. PhD in physics from University of Pittsburgh. Postdoctoral training at University of California San Diego. Dr. Liu and his team focus on the spatial and dynamical properties of biofilm, and mechanisms of biofilm resistance to antibiotics and to phage.

SELECTED ORAL PRESENTATIONS: ABSTRACTS

O1. *Staphylococcus aureus* Aggravates Atopic Dermatitis Through Enhanced Bacterial Colonization by Surface Protein

Nadira Nurxat[1,2,5,*], Na Zhao[1,5,*], Shucui Wang[3,5,*], Xilong Zhang[1,2], Yanan Guo[1], Qin Zeng[3], Xing Han[1], Hanlu Wang[1], Hua Wang[1], Yali Yang[4*], Min Li[1,2*], Ming Li[3*], Qian Liu[1,2*]

[1]Department of Laboratory Medicine, Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China. [2]Faculty of Medical Laboratory Science, College of Health Science and Technology, School of Medicine, Shanghai Jiao Tong University, Shanghai, China. [3]Department of Dermatology, Children's Hospital of Fudan University, National Children' s Medical Center, Shanghai 201102, China. [4]Department of Dermatology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200011, China. *These authors contributed equally.

Despite *Staphylococcus aureus* colonization as a recognized contributor to atopic dermatitis (AD) pathogenesis, the genetic mechanisms underlying *S. aureus* cutaneous colonization on AD-affected skin and its contribution to disease exacerbation remain incompletely understood. Through an integrated analysis of the microbiome and phenotypic analyses of clinical *S. aureus* isolates from pediatric AD cohorts, we established an association between bacterial biofilm proficiency and disease severity. Mechanistically, this hyper-biofilm phenotype is associated with a significant reduction in the activity of the SaeRS two-component system in *S. aureus*. The SaeRS system enables the bacterium to adapt to cutaneous stressors. Comprehensive transcriptomic and proteomic analyses demonstrate that SaeRS downregulation promotes biofilm biogenesis via coordinated upregulation of surface adhesin SdrC (serine-aspartate repeat protein C). We confirmed that SdrC enhances *S. aureus* adherence to epidermal keratinocytes, disrupts epithelial barrier integrity, and amplifies IL33-driven type 2 immune responses in a murine AD model. Collectively, our findings reveal a previously underappreciated role of the SaeRS–SdrC axis in mediating the interaction between *S. aureus* and the pathophysiology of AD, suggesting that targeting SdrC may represent a viable therapeutic strategy for managing this chronic inflammatory disorder.

O2. Utilising an *in-vitro* Biofilm Model of Chronic and Recurrent Middle Ear Infections to Investigate Antibody Function

Sharon Clark[1,2], Elke Seppanen[2], Lauren Bakaletz[3], Peter Richmond[1,2,4], Filippo Valente[1,5], Ruth Thornton[2,6]

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Chronic and recurrent otitis media is associated with persistence of non-typeable *Haemophilus influenzae* (NTHi) within biofilms in the middle ear, against which antibiotics are ineffective. Children experiencing recurrent acute episodes of otitis media, or chronic otitis media with effusion, are classified as otitis-prone, and are recommended for surgical intervention. Our previous work has shown that otitis-prone children have reduced natural antibody titres to several NTHi antigens involved in epithelial attachment and biofilm formation, compared with non-otitis-prone children. Here, we aimed to assess the functional consequence of these reduced antibody titres by measuring sera-mediated biofilm disruption using our *in-vitro* NTHi biofilm model. In our model, human middle ear epithelial cells were grown to confluency and fixed to provide a physiologically relevant substrate for NTHi biofilm formation. Mid-log phase NTHi were cultured in DMEM supplemented with 10% FCS and 30 mg/L hemin at 37 ˚C, 5% CO_2 for 96hrs to establish mature biofilms. Biofilm disruption was assessed by adding 10% pooled heat-inactivated sera from otitis-prone children (n=15 × 2 pools) or age-matched non-otitis-prone controls, together with 25% baby rabbit complement. Following incubation, total biomass was quantified using crystal violet staining. Biofilm disruption was calculated as the proportional reduction in biomass relative to untreated biofilms. Sera from non-otitis-prone children induced NTHi biofilm disruption (51%). In contrast, sera from otitis-prone children exhibited markedly reduced disruption, ranging from 0-31%, indicating impaired functional antibody activity. Sera from otitis-prone children show significantly reduced ability to disrupt mature NTHi biofilms compared with non-otitis-prone children, suggesting impaired antibody-mediated biofilm control. Further investigation is required to define the relationships between specific antibody titres, antigen targets, and functional biofilm disruption. These findings support a role for antibody-mediated protection against chronic and recurrent otitis media and highlight potential immunological targets for preventing disease.

O3. From Biofilm-Infected Mouse Burns to Diabetic Foot Ulcers: Translating Plasma-Activated Water for Chronic Wound Cleansing

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Chronic wounds such as diabetes-related foot ulcers are commonly driven by biofilm infections that are highly tolerant to antibiotics, creating an urgent need for antibiotic-free therapies that can reduce biofilm burden while supporting tissue repair. Plasma-activated water (PAW) is a liquid enriched in reactive oxygen and nitrogen species that shows rapid antimicrobial activity *in vitro* and in vivo, making it a promising biofilm-targeted wound rinse.
We established a translational pipeline to evaluate PAW from mouse biofilm infection through to pig wounds and a first-in-human trial. In a bioluminescent methicillin-resistant *Staphylococcus aureus* scald burn model in mice, wounds were infected to establish biofilm and treated with PAW or vehicle for 7 days. PAW significantly reduced bacterial bioluminescence from day 5 and lowered bacterial counts at day 8 by 2 logs ($p<0.05$), while improving re-epithelialisation without impairing wound closure, indicating simultaneous biofilm control and accelerated healing.
In a *Staphylococcus aureus*-infected excisional wound model in pigs, over 28 days PAW was compared with the clinical standard Prontosan (polyhexamethylene biguanide [PHMB]). Both treatments achieved similar reductions in bacterial load (1.5-2.5 logs, $p>0.05$), complete re-epithelialisation and collagen remodelling, with no local or systemic adverse effects, demonstrating equivalence to standard care and a favourable safety profile.
These data informed the design of an ongoing phase I double-blind randomised controlled trial in adults with low-risk diabetes-related foot ulcers, which is assessing safety and tolerability of PAW wound rinse compared with saline, with exploratory biofilm and healing outcomes.
Together, these studies show that PAW can reduce biofilm burden and support wound repair across species, providing a strong translational pathway toward clinical use in chronic biofilm infected wounds.

O4. Novel Quinazolinone-Based PqsR Antagonists as Quorum Sensing Inhibitors in *Pseudomonas aeruginosa*

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To overcome the continuously growing threats of infectious diseases and emerging pandemics, there is an urgent need to develop more effective alternative strategies to combat anti-microbial resistance, which represents one of the most serious challenges to global public health. *Pseudomonas aeruginosa* (PA) is a WHO-designated priority "superbug" responsible for a wide range of life-threatening infections, particularly in immunocompromised patients such as those with cancer and cystic fibrosis. It is well established that the quorum sensing (QS) systems in PA play a central role in the regulation, synthesis, and release of numerous virulence factors, including pyocyanin and biofilms. The *Pseudomonas* quinolone signal (PQS) system is one of the three major QS systems in PA. 2-Alkyl-4-quinolone-based autoinducers such as PQS and HHQ, bind to and activate the transcription regulator protein receptor PqsR (also known MvfR). Targeting PqsR with competitive antagonists represents a promising anti-virulence strategy to disrupt QS in PA and potentially mitigate antimicrobial resistance. To inhibit PQS-mediated quorum sensing in PA, we designed and synthesized a series of novel quinazolinone-based competitive antagonists of PqsR. We characterised their structure-function relationships and spectrum of antimicrobial activity[1-3]. The most potent analogue efficiently inhibited the PQS system exhibiting an IC_{50} of 4.5 μM in the PAO1 *pqsA:gfp* reporter assay. The lead compound completely suppressed pyocyanin production and significantly reduced biofilm formation. Furthermore, it showed synergistic activity in combination with known antibiotics at sub-MIC levels (1/2 MIC). Collectively, these findings highlight quinazolinone-based QS inhibitors as promising cost-effective candidates for the development of next-generation antimicrobial agents.

O5. Composition of the Uropathogenic *Escherichia coli* Biofilm Extracellular Matrix Impacts Resistance and Pathogenesis

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Uropathogenic *Escherichia coli* (UPEC) is the major cause of urinary tract infection (UTI). During infection, UPEC forms biofilms that enhance resistance to antibiotics and host immune factors. UPEC biofilm formation is a complex process involving adhesins and production of an extracellular matrix (ECM) comprising different combinations of curli amyloid fibres and the polysaccharide cellulose. These ECM components have different properties that influence infection outcomes, underscoring the need to understand their biological roles. Here, we show that UPEC infection severity is associated with compositional changes in the biofilm ECM, with UPEC strains that make curli (but not cellulose) causing augmented innate immune signalling and tissue neutrophil infiltration in a mouse model of UTI. *In vitro* examination of UPEC biofilms showed that production of an ECM comprising curli and cellulose generates significantly greater biofilm biomass compared to an ECM composed of curli alone. Both ECM types also provided significant protection against two cationic antimicrobial peptides (AMPs), human cathelicidin (LL-37) and polymyxin B, compared to planktonic cells. To define properties of UPEC biofilms that underlie AMP resistance, we employed single-plane illumination microscopy combined with fluorescence correlation microscopy (SPIM-FCS) to precisely quantify the diffusion of fluorescently-labelled molecules, including dextrans and AMPs. Both curli-only and curli+cellulose ECMs significantly inhibited the diffusion of inert dextrans (40-500 kDa) and AMPs, with the two ECMs exhibiting similar sieving capacity. Quantification of LL-37 diffusion also revealed a charge-based sequestering effect, where the diffusion of LL-37 was significantly reduced relative to a size-matched inert peptide. Treatment of biofilms with polymyxin B, but not LL-37, led to increased dextran diffusion, likely reflecting reduced biofilm density and structural integrity. Overall, our results demonstrate how composition of the UPEC biofilm ECM influences both host inflammatory responses and biofilm-associated antimicrobial resistance through size- and charge-dependent mechanisms.

O6. Outsmarting the Bladder: How the Biofilm Matrix of *P. aeruginosa* is More than Just a Protective Mechanism in the Urinary Tract Environment

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Background: *P. aeruginosa* accounts for 20% of all nosocomial UTIs, often associated with biofilm encrustation of indwelling catheters. Previous studies with *P. aeruginosa* indicate that urine influences downregulation of quorum sensing (QS), potentially increasing susceptibility to clearance by host immune system. Despite a lack of QS in urine, *P. aeruginosa* has adapted to thrive and establish biofilms in the urinary tract environment. In this study, we investigated the influence of urine on the *P. aeruginosa* biofilm matrix composition during UTIs and its immunogenicity to uroepithelial cells. Methods: Clinical uropathogenic *P. aeruginosa* biofilms were grown in the presence of pooled human urine or tryptic soy broth for 48 hrs at 37°C in slanting centrifuge tubes. The biofilm matrix was scraped and harvested using a series of gradient and chemical centrifugation steps. Protein, eDNA, polysaccharides and pyocyanin were quantified using standard methods. Mass spectrometry on the protein fraction defined urine mediated changes to the matrix proteome. To study the effect of the biofilm matrix on urothelial cellular inflammation, uroepithelial cell monolayers (ATCC 5637) were treated with different fractions of the harvested biofilm matrix without bacteria for 6, 12 and 24hrs. Pro-inflammatory cytokines and rates of wound healing were quantified. Results: Urine grown biofilm matrices showed a >3-fold increase in protein concentrations, and a significant reduction in polysaccharides ($p<0.01$), compared to nutrient broth controls. The protein fraction elicited the strongest inflammatory response from urothelial cells, with a significant

increase in IL-6, IL-8 and IL-1b in response to infection (>2-4 fold compared to untreated controls, $p<0.05$) and a significant reduction in rate of wound healing *in vitro*. Moreover, the protein fraction composition of urine grown biofilms was significantly different to TSB biofilms. No significant differences were recorded between the polysaccharide and eDNA fractions. Conclusion: Urine significantly influences the composition and immunogenicity of the *P. aeruginosa* biofilm matrix. The biofilm matrix has a larger protein component than extracellular polysaccharides compared to biofilms grown in nutrient broth. The matrix is also highly immunogenic to uroepithelial cells, indicating that its role in UTIs extends beyond bacterial protective mechanisms. Studies are currently underway to characterize the matrix proteome and biofilm transcriptome. This work highlights the importance of optimizing treatment options to target the biofilm matrix and reduce recurrent catheter infections.

O7. Ceftolozane/Tazobactam Plus Tobramycin Against Hypermutable *Pseudomonas aeruginosa* Epidemic Strains Investigated via a Dynamic Biofilm Model, Population Genomics and Mechanism-Based Modelling

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Introduction. Biofilm-associated *Pseudomonas aeruginosa* infections in cystic fibrosis (CF) have limited treatment options. We hypothesised that ceftolozane/tazobactam (C/T) plus tobramycin (TOB) combination regimens provide synergistic killing and resistance suppression of hypermutable *P. aeruginosa* epidemic strains (LES-1, CC274) from adolescents with CF. Methods. Isolates FQSE12 ($MIC_{C/T}$ 2mg/L, MIC_{TOB} 4mg/L, LES-1) and FQSE24 ($MIC_{C/T}$ 2mg/L, MIC_{TOB} 2mg/L, CC274) were investigated in 120-168h dynamic biofilm studies. The dynamic biofilm experiments simulated C/T and TOB lung pharmacokinetics representative of adolescents with CF. Regimens were: C/T 4.5g/day as continuous IV infusion ($penetration^{LungFluid}$=48%); TOB ($t_{1/2,LungFluid}$=3h, $penetration_{LungFluid}$=50%) 10mg/kg 24-hourly as 0.5h intravenous infusions; TOB ($t_{1/2,LungFluid}$=3h) 300mg 12-hourly inhaled; C/T + intravenous TOB; C/T + inhaled TOB. Total and resistant counts of planktonic and biofilm bacteria were determined. C/T and TOB exposures were confirmed by LC-MS/MS. C/T resistance mechanisms were investigated by whole-genome sequencing. Bacterial viable counts were mathematically modelled. Results. All monotherapies resulted in amplification of resistance emergence compared to the growth control, although inhaled TOB was more effective than intravenous TOB. C/T resistance development was associated with classical (AmpC overexpression plus structural modification) and novel (CpxR mutations) mechanisms depending on the strain. Against both isolates, the combination regimens demonstrated synergy and completely suppressed the emergence of C/T and TOB resistant planktonic and biofilm bacterial subpopulations. Mechanism-based modelling incorporating subpopulation and mechanistic synergy well described the antibacterial effects of all regimens against planktonic and biofilm bacteria. Parameter estimates from the mathematical model suggested longer mean generation times in the biofilm compared to the planktonic state of growth. Additionally, a lower maximum killing rate constant of TOB, and lower C/T concentration required for half-maximal inhibition of successful replication, were estimated for biofilm compared to planktonic bacteria, for both isolates. Conclusion. These findings support further investigation of C/T in combination with TOB against biofilm-associated *P. aeruginosa* infections.

O8. Pleural Infection and Biofilm

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Background: *Pseudomonas aeruginosa* uses c-di-GMP to regulate biofilm formation during pleural infections, and can induce neutrophils to form neutrophil extracellular traps (NETs). Our study investigates c-di-GMP-driven NETs generation in murine infection models.

Methods: mice were infected with *P.aeruginosa* strains expressing high (PAO1 △*wspF*), wild-type (PAO1), or low (PAO1/plac-*yhjH*) c-di-GMP levels to establish pleural infection. In vitro, c-di-GMP levels and biofilm formation of strains were verified using pcdrA-gfp reporter, crystal violet staining, and confocal microscopy. In vivo, pleural histopathology was observed via HE staining, confocal microscopy and scanning electron microscopy (SEM); RNA sequencing (RNA-seq) of mouse pleural lavage fluid cells was performed,
Results: In vivo, NETs detection revealed a C-di-GMP-dependent trend: PAO1 △ *wspF* induced the strongest NETs response (highest immune fluorescence intensity, cf-DNA, and MPO-DNA levels), followed by PAO1 and PAO1/plac-yhjH. RNA-seq and Mfuzz clustering identified 5 continuously downregulated gene clusters in infected mice; KEGG enrichment showed 48 genes enriched in the 'neutrophil extracellular trap formation ' pathway. PPI network analysis screened 10 hub genes (including Cybb, Mapk14, Pik3cd), whose differential expression was confirmed by qRT-PCR ($p<0.05$).

O9. Targeting Gastrointestinal Biofilms with Nature-Derived Antimicrobial Peptides

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Gastrointestinal (GI) disorders, such as inflammatory bowel diseases (IBD) and irritable bowel syndrome (IBS), affect ~6.8 million and ~780 million individuals globally, respectively.[1–5] Mucosal-associated biofilms are highly prevalent in the GI tracts of those patients.[6] Preliminary clinical work in removing these biofilms with endoscopic flushing reduces functional GI symptoms. Currently, no pharmaceutical interventions exist targeting such gut biofilms. Hence, we explored antibiofilm and antimicrobial peptides (AMPs) from diverse animal clades, including marsupials, monotremes, placental mammals, amphibians, and insects, for the development of oral peptide-based anti-gut-biofilm therapy.
We synthesised the AMPs via solid-phase peptide synthesis and screened against four biofilm-positive patient isolates of *Streptococcus parasanguinis* 102-K3/3, *Streptococcus salivarius* 102-K5/2, *Bacteroides fragilis* 93-K12 and *Escherichia coli* 104-K1 and two literature-control strains of *Staphylococcus aureus* ATCC29213 and *Pseudomonas aeruginosa* ATCC27853 by determining a minimum inhibitory concentration, and assessed antibiofilm activities using crystal violet staining and the Calgary device. We have also conducted structure-stability-activity relationship studies to enhance gut stability and potency of identified peptide leads for oral administration. Here, we showcase our latest results in developing oral anti-gut-biofilm peptide leads for the treatment of gastrointestinal disorders.

O10. Screening and Characterization of Natural Edible Antibiofilm Compounds against *Staphylococcus aureus*

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Natural edible compounds, derived from both medicinal and dietary sources, are emerging as promising alternatives to traditional antibiotics for controlling bacterial biofilms. In this study, we employed an integrated virtual screening and molecular docking approach to identify natural compounds with potent antibiofilm activity against *Staphylococcus aureus*. We then confirmed their antibiofilm efficacy and explored their mechanisms of action.
1.Thymol, a natural phenolic compound, significantly reduced biofilm formation, hemolytic activity, and staphyloxanthin production. These effects were attributed to its inhibition of FloA protein biosynthesis, a key component of functional membrane microdomains (FMMs).
2.Eugenol, another natural phenolic compound, exhibited a multifaceted profile, demonstrating antibiofilm, antioxidant, and antivirulence properties. These activities were linked to the modulation of purine/pyrimidine metabolism and the arginine biosynthesis pathway.
3.Additionally, 2-hydroxy-4-methoxybenzaldehyde, a bioactive constituent of Hemidesmus indicus, effectively removed nearly 80% of methicillin-resistant *S. aureus* (MRSA) biofilms. It also served as a potent antibiotic alternative by disrupting the bacterial cell membrane, leading to strong anti-MRSA activity.
Overall, this study identifies multiple natural edible compounds with distinct yet complementary antibiofilm mechanisms, providing new insights into the development of safe and effective anti-MRSA therapeutics.

O11. Biofilm Formation and Virulence-Associated Phenotypes in *Campylobacter concisus*: Investigating the Role of *CheB* and *luxS*

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Campylobacter concisus is a pathobiont of the human oral microbiota and increasingly implicated in human gastrointestinal tract (GIT) disorders. The molecular mechanisms of how *C. concisus* translocate from the oral cavity to various environmental niches and persist in exogenous sites remain poorly resolved. It is hypothesised that, like other mucosal pathogens, *C. concisus* may also exploit chemotaxis mechanisms to translocate to different human GI-sites. Furthermore, once found a favourable environment, *C. concisus* may initiate colonisation using various mechanisms, such as biofilm formation, cell adhesion, and invasion. Therefore, this study examines the contributions of *CheB*, a key chemotaxis regulator and *luxS*, involved in AI-2–mediated quorum sensing, to the virulence-associated traits in *C. concisus*. Phenotypic assessments between *C. concisus* RMIT-O17, wild-type (WT) strain, against mutant variants: *C. concisus* RMIT-O17Δ*CheB::Kan* and *C. concisus* RMIT-O17Δ*luxS::Kan* were conducted. A significant decrease in chemotaxis response was observed between the Δ*CheB* compared with WT (*** $p < 0.0005$), while no significant difference was detected between WT and the Δ*luxS* mutant. In contrast, chemotaxis differed significantly between the Δ*CheB* and the Δ*luxS* mutants (* $p < 0.05$) in a modified lab-based chemotaxis assay. In addition, Δ*CheB* and Δ*luxS* mutants exhibited a reduced biofilm formation ability. Preliminary cell adhesion and invasion assays suggest that Δ*CheB* and Δ*luxS* mutants have a significantly impaired ability to adhere and invade Caco-2 cells compared to WT. Together, these findings may demonstrate that quorum-sensing and chemotaxis pathways affect virulence-associated phenotypes through complementary yet mechanistically divergent routes. Hence, this mutagenesis study, together with laboratory-based assays, demonstrates that biofilm formation is controlled by a complex and interconnected cellular regulatory network. By delineating the roles of *CheB* and *luxS* genes, this study advances our understanding of *C. concisus*'s biology and offers insight into factors that may contribute to its persistence and pathogenic potential in GIT infections.

O12. Development of 5-Flucytosine Resistance in *Candida auris* During Combination Therapy is Associated with Reduced Virulence: A Case Report of In-Host Evolution

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Objective:
Antifungal combination therapy with 5-FC appears as a promising strategy for treating *C. auris* infections; However, we observed the development of resistant *C. auris* even under dual treatment. This study aims to characterize the evolutionary trajectories of resistance and virulence in the context of combination therapy , thereby informing clinical decision-making.
Method:
Antifungal susceptibility testing, whole-genome sequencing were performed on ten isolates obtained in a single patient case. Among these, a 5-flucytosine-susceptible isolate (CA001S) and a resistant isolate (CA016R) underwent phenotypic characterization, including biofilm formation assays, confocal laser scanning microscopy, and *Galleria mellonella* larval infection modeling. Comparative genomic analysis of CA001S and CA016R revealed their resistance and virulence mechanisms.
Result:
During combination therapy with voriconazole and 5-flucytosine for a *C. auris* urinary tract infection, the isolate acquired high-level 5-flucytosine resistance that concomitantly impaired its biofilm-forming capacity, resulting in a significant attenuation of virulence. Comparative genomic analysis identified a novel FUR1 frameshift mutation (FUR1_Leu13fs) that conferred 5-flucytosine resistance, and PLC mutation (PLC_Lys493Arg) and DUR32 mutation (Leu23His) involving virulence.
Conclusion:
Collectively, these findings illuminate the evolutionary trade-offs during antifungal treatment and emphasize the clinical imperative to monitor both molecular resistance markers and phenotypic virulence characteristics.

O13. Engineering *E. coli* Biofilms With Native and Heterologous EPS for Diverse Applications

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Enzymes capable of depolymerizing hydrolysable plastics have been discovered and engineered for industrial use in plastic recycling. Despite extensive protein engineering efforts to significantly increase the catalytic efficiency of plastic-degrading enzymes, inefficient enzyme-plastic interactions during this interfacial biocatalytic process continue to impede their widespread applications. Here, we use enzymatic PET degradation as a model system to describe a biofilm-assisted technique for enhancing enzyme interactions with insoluble substrates. By integrating the entire curli operon (*csgBACEFG*) into multiple chromosomal loci, we generated a super-adhesive, CsgBACEFG-optimized (CSGO) curli biofilm platform that promotes effective plastic surface adhesion and enzyme localization. FAST-PETase was embedded in the CSGO biofilm through the SpyTag/SpyCatcher system, resulting in 38-fold accelerated PET degradation activity when compared to planktonic cells. Additionally, PET film depolymerization revealed 2.5 times higher product formation using FAST-PETase-loaded CSGO biofilm compared to soluble FAST-PETase, and retained >50% activity after five reuse cycles in PET nanoplastic degradation. Accelerated PET-film degradation proved that the proposed concept of biofilm-confined FAST-PETases is preferred for this interfacial catalysis process, and that finetuning the biofilm thickness is an important parameter for efficient degradation. Collectively, this study establishes a modular, biofilm-bridged platform to enhance PET depolymerization, offering a generalizable approach for sustainable plastic recycling.
Key words: interfacial catalysis, biofilm, plastic degradation, PETase, curli, PET

O14. Mechanism of Mammalian Body-Heat Sensing by a Bacterial Thermosensory Diguanylate Cyclase

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In *Pseudomonas aeruginosa*, the intracellular second messenger cyclic diguanylate (c-di-GMP) regulates extracellular polysaccharide production, motility, and virulence. Our group has discovered the thermosensory diguanylate cyclase (TdcA), which synthesizes c-di-GMP at mammalian body temperature, modulating *P. aeruginosa* virulence traits. Temperature-sensing is mediated by a thermosensitive PER-ARNT-SIM (thermoPAS) domain that regulates an adjacent GGDEF domain for c-di-GMP synthesis; however, the mechanism of thermal sensing remains unknown. Here, we used integrative structural biology approaches, synthetic enzyme design, genetic analyses, and biochemical assays to define features essential for TdcA's temperature-dependent activity. Hydrogen-deuterium exchange mass spectrometry identified five heat-labile regions in TdcA: an N-terminal amphipathic region, the F and Jα-helices, and the G and Ib strands of thermoPAS. Cryo-electron microscopy and AlphaFold3 modelling were used to produce a preliminary structure of a 74 kDa TdcA dimer with local resolution as low as 3.74 Ångstroms. Bioinformatics identified the amphipathic N-terminus as a signature of TdcA orthologs. Mutagenesis of this region abolished temperature sensitivity *in vitro* and *in vivo*. Subcellular fractionation and immunoblotting revealed that TdcA is localized to the inner membrane by its N-terminus. Mutagenesis showed that regions beyond the PAS core, including the N-terminus and linker, are essential for thermal activation. Linker mutations locked the enzyme "on" or "off." A synthetic β-galactosidase bifurcated with thermoPAS

displayed temperature-dependent activity when thermoPAS remained intact and properly positioned. Cross-linking MS supported temperature-dependent rearrangement of the N-terminal amphipathic region. Taken together, we propose that TdcA functions as a membrane-coupled thermosensory device. Temperature-dependent structural changes are transmitted through an axis originating in a membrane-embedded, N-terminal amphipathic helix, through the PAS domain and linker, and terminating in the GGDEF domain to reorient the catalytic site of a dimer. These findings reveal modular accessory elements to the canonical PAS domain that are fundamental to thermal sensation in TdcA.

O15. Unconventional Strategies Promote the T6SS-Mediated Fitness in Polymicrobial Communities

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Biofilm communities are not static assemblies but highly dynamic ecosystems shaped by continuous cooperation, competition, and spatial restructuring. Within these dense, multicellular environments, microbes deploy specialized mechanisms to eliminate competitors, construct community architecture, and secure local niches. Among these mechanisms, the type VI secretion system (T6SS) is one of the most powerful drivers of biofilm organization. Many Gram-negative pathogens utilize the T6SS to inject diverse toxic effectors into neighboring bacterial and fungal cells. T6SS activity therefore plays a central role in shaping the spatial patterning of biofilms, determining which strains persist, which are displaced, and how community structure evolves over time. Despite its ecological importance, the mechanisms that ensure precise assembly, activation, and coordination of cytosolic and membrane-associated T6SS components remain poorly understood. In this talk, I will present recent mechanistic insights into how effector proteins and key assembly initiators orchestrate T6SS biogenesis and firing. I will further discuss how these unconventional regulatory principles impact interspecies interactions and biofilm dynamics.

Bio： Dr. Tao Dong is a professor and associate dean in the School of Life Sciences at the Southern University of Science and Technology. He obtained his BSc from Shandong University in 2003 and his PhD from McMaster University in 2010. Funded by Banting Fellowship, he worked with John Mekalanos at Harvard Medical School from 2011 to 2013. Before joining SUSTech, he served as a full professor and Canada Research Chair in Molecular Ecology of Waterborne Microbes at the University of Calgary. He also served as department chair of Biochemistry and Molecular Biology at Shanghai Jiao Tong University. Dr. Dong's research focuses on understanding the molecular adaptation mechanisms that enable bacteria to survive in diverse natural and host environments and the nature of bacterial interactions with competing species. He currently serves as an editor for the Journal of Bacteriology and as editorial board member of mLife and iMeta.

O16. Lindo Drain: Prevention of Biofilm Formation in Healthcare Drains

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Sink and floor drains in healthcare settings frequently harbour complex, multilayer biofilms that include opportunistic and multidrug resistant organisms. These reservoirs can disseminate upward through splashing and aerosols generated during routine basin use, creating a persistent route of contamination that is difficult to manage with existing cleaning practices. Conventional mitigation relies on intermittent thermal and chemical interventions. Current approaches include prolonged hot water flushing, boiling water disinfection applied after pre-warming the drain, and short duration steam followed by hot water flow. Chemical methods commonly involve dosing chlorine solutions with defined contact times or applying hydrogen peroxide based foams every few days to the upper drain region. While these techniques can temporarily reduce culturable organisms, recolonisation within days is common and the labour required to maintain these regimes limits long term effectiveness.
Lindo Drain is a new platform under development intended to provide continuous, low maintenance suppression of drain associated biofilms. The system couples an external light engine to a purpose designed optical sleeve that delivers controlled emission of antimicrobial blue light into the water column and wetted surfaces of the trap. The design enables targeted delivery of multi wavelengths to stimulate endogenous bacterial photosensitisers, generating reactive oxygen species capable of disrupting both wet and intermittently dry biofilm layers. The optical architecture incorporates defined light delivery regions engineered to maintain consistent radial emission under varying moisture conditions.

Laboratory studies using the same multi wavelength antimicrobial blue light engine have demonstrated multi log reductions across representative Gram positive and Gram negative species in controlled carrier tests and simulated wastewater models. These findings form the basis for Lindo Drain's development pathway.
At the time of submission, in situ deployment in operational healthcare sinks has not yet commenced. This work therefore outlines the design rationale, summarises relevant laboratory evidence, and describes a planned evaluation programme to assess continuous antimicrobial blue light as a complementary strategy for sustainable control of drain associated biofilms.

O17. *In Vitro* Biofilm Formation at the Gut-Mucus Interface under Flow Conditions

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Microbial communities in the human intestine strongly influence health. They affect nutrient metabolism, digestion, vitamin synthesis, and immune system regulation. Colonic microbial communities are highly structured within the mucus layer that lines the epithelium, where they are simultaneously exposed to mucus secretion and lumen hydrodynamic forces. However, the dynamic mechanisms of bacterial attachment, their potential transition into biofilms, and ultimately their invasion through the mucus remain poorly understood. This is largely due to the lack of physiologically relevant experimental models. Here, we present a novel microfluidic device designed to simulate key features of the microbiome-mucus interface under flow conditions. The device is based on the co-injection of mucus flow and bacteria flow in a confluence-based setup capable of generating a mucus gradient. Time-lapse microscopy of *Pseudomonas aeruginosa* revealed preferential bacterial accumulation and biofilm formation at the mucus interface, while minimal attachment was observed when buffer was used instead of mucus. Notably, biofilm formation persisted despite continuous mucus flow, indicating that a combination of mucus viscoelasticity, hydrodynamic trapping, metabolic effects, and shear stress mechanisms promotes retention at the interface.This microfluidic model provides a physiologically relevant platform to systematically study how flow, shear stress, mucus properties, and bacterial traits influence mucosal colonisation. By uniquely focusing on biofilms, this work provides new insight into microbial organisation in the gut and bridges an important gap between static culture models and the complex *in vivo* gut environment.

O18. Plasma-Activated Water, An Egg-streme Clean

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Biofilm formation on eggshell surfaces poses a food safety concern, enabling the persistence and cross-contamination of bacteria. This study evaluated the anti-biofilm efficacy of plasma-activated water (PAW), a liquid rich in reactive oxygen and nitrogen species (RONS) generated when plasma, the fourth state of matter, interacts with water. 50 bacterial isolates were recovered from chicken eggshells, and the biofilm-forming abilities of eight representative species (*Staphylococcus arlettae*, *Lysinibacillus fusiformis*, *Priestia megaterium*, *Peribacillus frigotolerans*, *Acinetobacter lwoffi*, *Escherichia coli*, *Pseudomonas fulva*, and *Pseudmonas stutzeri*) were characterised. All isolates produced biofilms with varying culturable cell populations, biomass, and extracellular polymeric substance (EPS) composition. EPS analysis of each isolate's biofilms identified polysaccharides, eDNA, and proteins as components of their matrices, with their relative abundance shifting with biofilm age. Importantly, PAW was tested

under short exposure times (1, 5, and 15 min) using freshly generated, 1-week-old, and 4-week-old PAW. Fresh PAW exhibited potent anti-biofilm activity, achieving complete inactivation below the limit of detection of *E. coli* and *P. stutzeri* biofilms within 1 min, while Gram-positive biofilms were generally more tolerant. Reduced efficacy of aged PAW correlated with the decay of RONS. Mechanistic analyses, including scanning electron microscopy and intracellular reactive oxygen species detection assays, linked PAW activity to biofilm disruption and intracellular accumulation of reactive species. This study provides the first detailed evaluation of biofilm formation by critically under-investigated eggshell-associated bacteria and elucidates the anti-biofilm mechanisms of PAW against these bacteria. We present PAW as an effective sanitising agent for the egg industry, supporting safer food production and reduced dependence on conventional disinfectants amid global surges in antimicrobial resistance.

Key Words: Biofilm, Plasma-Activated Water, Decontamination, Eggs, Food Sanitiser, Food Safety

O19. Identification of Persistent *Listeria monocytogenes* Strains in Enoki Mushrooms and Genetic Basis of Their Biofilm-Forming Capability

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Listeria monocytogenes is a prevalent foodborne pathogen contaminating the enoki mushroom industry. Its ability to form biofilms enhances bacterial persistence in the environment, leading to recurrent product contamination. Our study analyzed over 500 *L. monocytogenes* strains from China's enoki production chain, identifying ST8, ST87, and ST9 as persistent strains across four food enterprises through multilocus sequence typing (MLST). Biofilm assays demonstrated that these persistent strains formed more robust biofilms, with their capacity correlated to stress resistance. Genomic analysis of 103 strains identified the *vip* gene as a predictive marker for biofilm formation, with enhanced accuracy when combined with the *srmB*, *cycB*, and *uvrB* genes, as well as specific ST types. Furthermore, plasmid carriage was significantly correlated with biofilm formation; the removal of plasmids resulted in a 20-80% reduction in biofilm production, impairing both aggregation and extracellular protein secretion. Multi-omics analysis revealed that plasmids down-regulate carbohydrate metabolism, leading to a decrease in extracellular polymeric substance (EPS) levels. These findings provide genetic markers for the detection and control of *L. monocytogenes* in enoki production.

Keywords: Listeria monocytogenes, biofilm formation, persistence, genetic markers

O20. Antibiotic Selection Pressure Remodels Biofilm Metabolism and Architecture to Promote Horizontal Resistance Gene Transfer

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Horizontal gene transfer (HGT) is a central ecological process driving the spread of antimicrobial resistance (AMR) within microbial communities. Biofilms, the predominant mode of bacterial life in natural and host-associated environments, are characterized by strong spatial structuring, metabolic heterogeneity, and close intercellular interactions, all of which can influence gene flow. However, how antibiotic selection pressure alters biofilm ecology to modulate resistance dissemination remains poorly understood.
Here, we show that sublethal streptomycin exposure significantly enhances conjugative transfer of resistance genes from *Salmonella* to *Escherichia coli* within dual-species biofilms. Antibiotic selection pressure induces broad shifts in the extracellular metabolic landscape, accompanied by coordinated metabolic activation within specific biofilm subpopulations. These ecological responses coincide with pronounced changes in biofilm architecture and donor–recipient spatial organization, collectively increasing the probability of effective cell-to-cell contact and plasmid transfer.At the community level, antibiotic exposure reshapes population dynamics and assembly within murine fecal

microbiota. Resistance genes are preferentially maintained and disseminated among closely related Enterobacteriaceae, consistent with ecological filtering. Notably, antibiotic selection also facilitates early dissemination to more phylogenetically distant taxa, indicating that antibiotic stress can relax phylogenetic constraints on HGT and expand the ecological host range of resistance determinants. Here, we show that sublethal streptomycin exposure significantly enhances conjugative transfer of resistance genes from *Salmonella* to *Escherichia coli* within dual-species biofilms. Antibiotic selection pressure induces broad shifts in the extracellular metabolic landscape, accompanied by coordinated metabolic activation within specific biofilm subpopulations. These ecological responses coincide with pronounced changes in biofilm architecture and donor–recipient spatial organization, collectively increasing the probability of effective cell-to-cell contact and plasmid transfer. At the community level, antibiotic exposure reshapes population dynamics and assembly within murine fecal microbiota. Resistance genes are preferentially maintained and disseminated among closely related Enterobacteriaceae, consistent with ecological filtering. Notably, antibiotic selection also facilitates early dissemination to more phylogenetically distant taxa, indicating that antibiotic stress can relax phylogenetic constraints on HGT and expand the ecological host range of resistance determinants.

O21. When Food Goes Bad: Studying Biofilm Formation to Reduce Food Spoilage and Extend Shelf Life

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Almost 10% of the world's population do not have enough to eat. Despite this ~20% of all food produced for human consumption worldwide is wasted, with food spoilage by microbes being a key contributor to this wastage. The microbes that cause spoilage grow on food surfaces in biofilms. While single-species studies have facilitated some mechanistic understanding of how these biofilms develop to cause food spoilage, native food microbiomes are multi-species. Additionally, while there is some knowledge of microbiome composition, we have a poor understanding of how individual members interact with one another and the food surface during spoilage biofilm development. Here we aim to obtain a detailed molecular mechanistic understanding of spoilage biofilm development by the native community on two food commodities, chicken breast and baby spinach. We are using a range of approaches including full-length 16S rRNA sequencing, metabolomics, metatranscriptomics, fluorescence microscopy and molecular biology. Our data thus far has determined the composition of the chicken and spinach microbiomes at the species level, as well as the associated metabolomes, across the spoilage timeline. Visualisation of biofilm development on both commodities has revealed that tissue destruction is associated with spoilage biofilm formation. Remarkably, the biofilms formed on chicken are composed of pillar-like tunnels that extend into the tissue, which in many cases are associated with extracellular DNA (eDNA). We are currently investigating whether this eDNA is host- and/or microbe-derived and determining the relative abundance of different eDNA conformations (e.g. B-DNA, Z-DNA and G-quadruplex). We are also developing synthetic communities (SynComs) to facilitate more in-depth mechanistic studies of in situ community interactions and biofilm development. Our long term aim is to use the fundamental understanding we obtain to develop novel interventions to target spoilage biofilms to increase food shelf life and reduce food waste.

O22. Acclimation-Enhanced Formation of Self-Immobilised Biofilms (Aerobic Granules) for Improved Wastewater Treatment

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Aerobic granules act as self-immobilised biofilms that provide compact structure, enhanced settling, and efficient nutrient transformation within wastewater microbiomes, making the conditioning of seed sludge a crucial step in promoting early biofilm-based granulation. This study investigated the impact of short-term acclimation on biomass fitness and initial aerobic granule development in lab-scale sequencing batch reactors (SBRs). Seed sludge sourced from a municipal wastewater treatment plant was acclimated for three days in 50%-strength synthetic wastewater containing ammonium sulfate, phosphate buffers, and sodium acetate under continuous aeration (2.4 cm/s). Acclimation improved sludge compaction and aggregation potential, with settling volume decreasing from 880 to 830 mL/L, total suspended solids (TSS) increasing from 5.85 to 7.14 g/L, and sludge volume index (SVI) improving from 151 to 116 mL/g, indicating tighter microbial

clustering and enhanced biofilm-forming capability. The acclimated biomass was then inoculated into three SBRs operated on 2-hour cycles, where hydrodynamic selection imposed by short settling periods and substrate gradients during feeding facilitated rapid biofilm-driven granulation. Compact spherical aggregates of 1–2 mm formed within 20–21 hours, significantly earlier than the ~30-hour granulation typically observed using non-acclimated sludge (SVI 176.2 mL/g, TSS 4.74 g/L). These findings demonstrate that acclimation enhances microbial physiological readiness for aggregation, accelerating the establishment of self-immobilised aerobic granules under short-cycle operation. The improved structural integrity and early biofilm formation observed in acclimated sludge highlight its potential for strengthening biological wastewater treatment processes by promoting more resilient and efficient microbial communities. This work provides insight into how controlled pre-conditioning can optimise microbial aggregation mechanisms, supporting the application of engineered and naturally formed biofilms for enhanced wastewater bioprocess performance.

O23. Seagrass Holobionts Through the Seasons: Ecology Under Environmental Stress

Tamar Jamieson, Mohsen Chitsaz, Angélique Gobet, Michelle Waycott, Sophie C. Leterme

Microbial communities are widely recognised as indicative of ecosystem health. Changes in the microbial community composition of seagrasses and their environment could act as an important bio-indicator for stress factors affecting the submerged aquatic plants that make up the Ruppia community in the Coorong. Here, we explored prokaryotes associated with surface biofilms of the leaves and roots of the seagrasses to determine the microbiota composition of the Ruppia community, and their link to the surrounding sediment and water. Ruppia was recorded growing at 55% of the sites surveyed, and all collected samples showed a high diversity of prokaryotes. Turbidity was the main driver of the fluctuations in microbiota composition of the Ruppia community. Water and sediment microbial communities were correlated with the presence/absence of the seagrasses. Seagrass health indicators were assessed, allowing for a clear distinction between the various states of the Ruppia community identified in this study. This study provides key baseline insights into the composition and possible functions of these biofilm microbiota, as well as identifying potential health bio-indicators for the Ruppia community. Furthermore, it identifies specific beneficial bacteria that could be selected to enhance seagrass restoration efforts as well as inhibit detrimental algal blooms in the Coorong.

O24. From Fouling to Biofilms: Exploring Microbial Community Assembly and Corrosion Behaviour in Confined Marine Microenvironments

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Confined microenvironments, such as those created beneath macrofouling organisms, offer ideal conditions for biofilm establishment and community assembly. Yet the factors governing microbial colonisation patterns, community structure and phylogenetic diversity within fouling-induced crevices remain poorly characterised. Understanding how physical confinement shapes biofilm community assembly in varying marine environments is essential for predicting microbial behaviour in natural and industrial marine systems. This study used mechanical fouling analogues and CuNi alloys, widely used in marine infrastructure, as a controlled model to investigate how macrofouling-like structures influence biofilm development and associated material corrosion in real marine environments. Two analogue types were designed: (1) hard fouling analogues with defined crevice gap widths (50 µm to 1 mm) to mimic barnacle- or mussel-type attachments, and (2) soft fouling analogues using porous sponge materials to reproduce the more diffuse, fluid-permeable microenvironments of soft-bodied macrofoulers. CuNi 90/10 alloy coupons with attached analogues were deployed at temperate (Melbourne) and tropical (Cairns) marine sites for varying exposure durations. Post-exposure characterisation included 3D profilometry of localised corrosion and microbial community profiling to characterise phylogenetic diversity and taxonomic composition. The results reveal distinct microbial taxa and phylogenetic diversity between analogue types and environments, reflecting differential biofilm assembly under contrasting physical and chemical conditions. Localised corrosion patterns also varied across analogue types and sites suggesting complex interactions between biofilm communities and material behaviour. This work establishes an experimental framework to investigate biofilm assembly, community structure and material responses in spatially confined, field-relevant environments, advancing knowledge of how physical and environmental factors drive biofilm ecology in industrial and natural marine systems.

O25. Free Chlorine Enhances Bacterial Invasion and Conjugative Transfer of Antibiotic Resistance Genes in Biofilms

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Biofilms underpin persistent microbial survival across environmental and industrial systems. The dense, spatially structured architecture creates ideal niches for cell-to-cell interactions that enable horizontal gene transfer. At Asia-Pacific Biofilms 2026, where the scope spans environmentally relevant biofilms and mechanistic biofilm biology alongside technologies to control biofilms, understanding how disinfection perturbs the relationship between biofilm structure and function is significant. Here, we investigated how free chlorine reshapes biofilm architecture and alters plasmid-mediated conjugative transfer of antibiotic resistance genes (ARGs) in drinking water biofilm models. Using mono- and multi-species biofilms composed of *Escherichia coli*, *Pseudomonas putida*, and *Pseudomonas aeruginosa*, we quantified biofilm disruption, donor invasion, and depth-resolved conjugation outcomes under increasing free chlorine exposure. We found that free chlorine at 5 mg/L enhanced plasmid-mediated conjugation frequencies by 26-fold compared with untreated controls in *E. coli* biofilm. In contrast, multi-species biofilms exhibited lower overall conjugation frequencies but still showed more than a 10-fold increase under free chlorine exposure, reflecting the competitive and spatially structured nature of interspecies interactions. Correspondingly, the spatial distribution of conjugation events shifted from surface layers (18 ± 2 μm) to deeper regions (27 ± 3 μm) in multi-species biofilm model, indicating a reorganisation of ecological interactions within the biofilm under free chlorine stress. Imaging and cultivation showed that free chlorine markedly disrupted biofilm architecture, providing optimal niches for deeper donor colonisation, thereby increasing contacts between cells and further enhancing conjugation. To resolve longer-term consequences beyond experimental windows, we developed a mathematical model capturing invasion and conjugation dynamics in structured biofilms under free chlorine. Simulations corroborated that 5 mg/L free chlorine promotes deeper donor penetration and broader ARG dissemination throughout the biofilm over a 7-day period. These findings link disinfection-driven biofilm structural disruption to bacterial invasion and accelerated horizontal ARG transfer, advancing our understanding of how disinfection practices may inadvertently influence antibiotic resistance dissemination in water distribution systems.

O26. Mimicking in vivo Infection Dynamics: A Physiologically Relevant 3D Platform to Study Biofilm-Related Infections

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In vitro biofilm models have advanced our understanding of biofilm formation, persistence, and antimicrobial tolerance, yet they fail to replicate the host microenvironment, physiological conditions, and biofilm–host interactions observed *in vivo*. To address this limitation, we developed a modular 3D platform that supports *Staphylococcus aureus* biofilm formation within gelatin methacryloyl(GelMA) hydrogels, an extracellular matrix mimic capturing key biochemical and mechanical features of soft tissues. *S. aureus* embedded in 5%GelMA were cultured either in minimum essential medium with 10% fetal bovine serum (MEM) to mimic host-like nutrients or in tryptic soy broth (TSB), the standard biofilm medium. Confocal imaging showed that single cells aggregated and proliferated into clusters that recapitulated the major biofilm hallmarks: aggregation, matrix deposition, maturation, and dispersal. Live/Dead imaging revealed viable clusters enriched in eDNA, while DAPI/WGA/ConA staining confirmed progressive polysaccharide-rich matrix deposition, with PIA/PNAG detectable from 3h and increasing through day14. Cryo-SEM demonstrated nutrient-dependent differences: larger TSB-grown clusters contained abundant cellular debris consistent with eDNA-mediated stabilization, whereas MEM-grown clusters consisted of healthy dividing cells embedded in ECM-like material and displayed abundant extracellular vesicles, suggesting EV-associated virulence under host-like conditions. Proliferation assays showed growth in both media but consistently higher bacterial numbers and metabolic activity in TSB. Mechanical testing indicated significant GelMA degradation only in TSB cultures, suggesting toxin-

and protease-mediated matrix remodelling. Untargeted metabolomics revealed distinct metabolic landscapes, with fast-growing, energy-intensive communities in TSB versus a survival-oriented phenotype in MEM that more closely reflects in vivo infection states. Finally, incorporating polycaprolactone (PCL) scaffolds to model implant–tissue interfaces showed that implants did not enhance bacterial proliferation or metabolic activity. Although bacteria migrated onto PCL surfaces via cluster dispersal, they did not establish robust surface biofilms after 7 days. Overall, this 3D platform provides a physiologically relevant system for studying biofilm-related infections and informing clinically meaningful anti-biofilm strategies.

O27. A Biofilm's Journey from Gut to the Brain: Microbial Biofilms in the Gut Have a Role in Human Ageing and Dementia Pathology

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Aggregation of proteins into fibrils – called amyloids, is a ubiquitous phenomenon that is involved in human ageing and dementia pathology. Why certain people develop dementia earlier or age faster than others, is not just genetics. Recent evidence suggest that gut-microbiome has a role in human health and disease. However, how gut-microbes play this role at molecular level is not explored. Infectious microbes in the gut make similar amyloids to support biofilm formation. These microbial amyloids are underlying structural unit of their biofilms. Our research has demonstrated that microbial biofilms in the gut can access the human brain through Gut-Brain Axis. By using a combination of models including transparent zebrafish, mice and human derived organoids, we have studied that microbial amyloids formed in the biofilms of opportunistic gut-pathogens (*Pseudomonas aeruginosa* & *E. coli*) can access the human brain and interact with Aβ. Aβ is a key protein in the brain that aggregates and make amyloids during human ageing and pathogenesis of Dementia. We have observed that interaction of gut-derived microbial amyloids with Aβ results in faster and toxic aggregation of Aβ, accelerating cognitive decline and neurodegeneration. On the Brain-to-Gut side of this journey, we have found that Aβ in the brain is produced in response to microbial biofilms in the gut. Aβ was traditionally considered a functionless peptide produced in the brain. We observed that, Aβ produced in the brain was able to translocate to the side of microbial biofilms, dissolved microbial biofilms and increased the efficiency of antibiotics. This revealed a native defensins-like role of Aβ against microbial biofilms in the gut. Overall, this research shows a bidirectional cross-talk between gut-microbial biofilms and human brain which corelates with brain ageing and dementia pathology through Gut-Brain Axis.

O28. Computational, Chemical Synthesis, and Biological Evaluation of Novel Peptidomimetics Against Multi-Drug Resistant Bacteria

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The accelerating global antimicrobial resistance (AMR) crisis has rendered many conventional antibiotics increasingly ineffective, underscoring the urgent need for novel therapeutic agents and alternative antimicrobial strategies. In this study, we report the rational design, synthesis, and biological evaluation of a series of guanidine-substituted anthranilic amide peptidomimetics incorporating an adamantane moiety as potential antibacterial agents against multidrug-resistant pathogens. An integrated computational workflow comprising molecular docking, molecular dynamics (MD) simulations, steered molecular dynamics (SMD), ADMET profiling, phylogenetic comparison with previously reported peptidomimetics, and density functional theory (DFT) analysis was employed to identify promising candidates prior to chemical synthesis. Among the evaluated compounds, molecular docking identified TP88 as a promising candidate. Further analysis showed that TP88 exhibited a higher fraction of sp^3 carbons (Fsp^3 = 0.40), indicating increased structural complexity and a potential improvement in pharmacokinetic behaviour. The compound also displayed slightly lower logP values, suggesting enhanced solubility and favourable drug-likeness. SMD simulations demonstrated that TP88 undergoes favourable conformational adaptation during membrane translocation, facilitated by the adamantane group, indicating an enhanced ability to penetrate bacterial membranes. Subsequent biological evaluation revealed potent antibacterial activity of TP88 against clinically relevant strains, including *Staphylococcus aureus* (ATCC 25923, MRSA, S. aureus strain 38, and *S. aureus* strain 15), with MIC values ranging from 0.95 to 7 μM, as well as *Escherichia coli* (MIC = 12–15 μM) and *Acinetobacter baumannii* clinical isolates (MIC range = 3.9–7 μM). Furthermore, time-kill kinetics and antibiofilm assays demonstrated rapid bactericidal activity and significant biofilm inhibition,

highlighting the therapeutic potential of these peptidomimetics. Collectively, this integrative approach demonstrates TP88 as a promising lead compound for the development of next-generation antimicrobials targeting multidrug-resistant bacteria.

Keywords:
Antimicrobial resistance; Peptidomimetics; Guanidine-substituted anthranilic amides; Adamantane; Molecular docking; Molecular dynamics simulations; Steered molecular dynamics; ADMET profiling; Density functional theory; Antibacterial activity; Multi-drug resistant bacteria; Biofilm inhibition.

O29. Modulation of Multibacteria and Polybacteria of Dental Biofilms Using an In-Situ Gelling System Loaded with Cyclo-L-Phe-L-Pro Derived from Endophytic Actinomycetes

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The oral cavity harbours nearly 700 bacterial species, and the formation and persistence of dental biofilms play a central role in the development of dental caries. An in-situ gelling system incorporating natural antibiofilm compounds represents a promising strategy for caries management by enabling prolonged retention and localized delivery of bioactive agents at the tooth surface. Endophytic actinomycetes, which reside within plant tissues, are recognized as prolific producers of biologically active secondary metabolites. This study aimed to evaluate the antibiofilm efficacy of a natural compound, cyclo-L-phe-L-pro, derived from endophytic actinomycetes and formulated into an in-situ gelling system, in comparison with a chemical antiseptic control (chlorhexidine), against polybacteria and multibacteria dental biofilms. Crude extracts from endophytic actinomycetes isolated from *Hopea ferrea* were prepared using tryptic soy medium and subjected to compound isolation and characterization. High-performance liquid chromatography (HPLC) was employed for profiling, followed by recycling HPLC for purification. Structural elucidation and isomer determination were conducted using nuclear magnetic resonance spectroscopy. Following isolation, the identified compound was incorporated into an in-situ gel formulation for antibiofilm testing. Polybacteria and multibacteria biofilm models comprising *Streptococcus mutans*, *Streptococcus oralis*, *Actinomyces viscosus*, *Actinomyces oris*, *Lactobacillus acidophilus*, and *Candida albicans* were developed. Biofilm biomass reduction was assessed using the crystal violet assay, while metabolic activity was evaluated using Cell Proliferation Kit II. Results demonstrated that the in-situ gel containing cyclo-L-phe-L-pro significantly inhibited biofilm biomass formation by up to 50% in both polybacteria and multibacteria models. Additionally, at 1 mg/mL, peptide formulations enhanced cell proliferation by approximately 6–8 fold compared to control, with preserved bioactivity following gel incorporation. These findings highlight endophytic actinomycetes as a valuable source of natural antibiofilm agents and support the potential application of peptide-loaded in-situ gels as an early preventive strategy for managing oral biofilm-associated diseases and reducing systemic health complications.

Keywords: Endophytic actinomycetes, multi-species dental biofilm, cyclic dipeptides, antibiofilm, biofilm biomass, cell proliferation.

O30. Mucin-Driven Streamer Biofilms in Gut-mimic Microfluidic Models

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Biofilm formation within the gastrointestinal tract occurs in a highly dynamic, mucus-rich environment, where host polymers and microbial secretions coexist under continuous flow. While mucin is widely recognised for its role in surface protection and microbial adhesion, its contribution to the bulk physical organisation of microbial communities under flow remains poorly understood. Here, we investigate how mucin modulates the formation of bacterial streamers within curved microfluidic channels, focusing on the emergence of large-scale polymeric networks rather than classical surface-attached biofilms. Using *Pseudomonas aeruginosa* and a 3D-printed microfluidic chip, we demonstrate that mucin dramatically accelerates streamer formation and increases structural stability under flow. In mucin-free conditions, streamer development is delayed and mechanically fragile, whereas the presence of mucin promotes rapid assembly of thick, cohesive networks within 40 minutes. Comparative experiments with synthetic

polymers of similar concentration, including polyethylene oxide, methylcellulose, and xanthan gum, failed to reproduce this effect, indicating that mucin's role extends beyond bulk rheological modification. Imaging wild-type bacteria and multiple biofilm knock-out mutants suggests that streamer formation does not rely on a single bacterial adhesin or exopolysaccharide, but instead emerges from physical interactions between mucin and a nascent bacterial polymer backbone. Together, our findings reveal a new role of mucin in shaping biofilm-like structures. This work introduces host–microbe polymer interactions as key physical determinants of gut microbial organisation relevant to physiology and disease.

SELECTED POSTER PRESENTATIONS: ABSTRACTS

P1. Effect of Temperature and Nutrients on Reptile Associated *Salmonella* Biofilm Formation

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The presence of *Salmonella* in reptiles poses a significant risk of transmission to humans. There is a growing interest in keeping exotic pets such as captive pythons in Australia. Reptile enclosures generally consist of a basking spot temperature at ~37˚C, alongside a cooler area maintained at ~25˚C. Our study investigated the effect of temperature (25˚C and 37˚C) and nutrient availability (full-strength and half-strength tryptic soy broth [TSB]) on monospecies biofilm formation of reptile associated *Salmonella* isolated from captive pythons. These included subspecies *enterica* (n=7), subspecies *diarizonae* (n=2) and subspecies *salamae* (n=1). The subspecies *enterica* serovars, were *S*. Jangwani (n=1) *S*. Java (n=1) *S*. Muenchen (n=2), *S*. Havana (n=2) and *S*. Umbadah (n=1). *S*. Typhimurium (ATCC 13311 and 14028) were used as reference strains in this study. Biofilms of these strains were grown on polystyrene surfaces using the culture-based (coupons) and microtitre plate technique for 24 h. Biofilm cell numbers and specific biofilm formation (SBF) across all strains were ~ 5.59–7.30 log CFU cm^{-2} and 0.03–3.08 respectively in full strength TSB. In half strength TSB, numbers and SBF were ~4.82 – 7.47 log CFU cm^{-2} and -0.05–5.54 at both temperatures. In full-strength and half strength TSB, most strains exhibited similar biofilm cell numbers / SBF at 25˚C and 37˚C, with no significant differences ($p \geq 0.05$). *S*. Java S2A and *S*. Typhimurium ATCC 14028 were the only strains that exhibited significantly ($p < 0.05$) higher biofilm cell numbers at 37 ˚C compared to 25 ˚C in both full strength and half strength TSB. All reptile-isolated strains were weak biofilm formers. Strains belonging to subspecies *enterica* were generally poorer biofilm formers than strains belonging to subspecies *diarizonae* and *salamae*. These findings may suggest that temperature and nutrient stress influence reptile-associated *Salmonella* biofilms, highlighting the potential significance of hygiene practices during reptile handling.

P2. Bacterial Pathogens and Harmful Assemblages Selected on Plastic Biofilms Impose Environmental Health Risks to Coastal Waters

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Plastic pollution in aquatic environments poses significant concerns and risks due to its potential to serve as a mobile biofilm substrate and refuge for aquatic pathogens, harmful algae, and other biological pollutants, such as antibiotic resistance genes, to disperse. However, the role of plastic surfaces and how biofilm interfaces may facilitate the selection of pathogenic and harmful biological components from the environment remains poorly understood. To address this gap, we employed environmental metagenomics using laboratory microcosms and field in-situ incubations in mariculture waters over a one-month period to investigate the relationship between plastic types, microbial communities, harmful microorganisms, and ARGs in the formation and succession of plastic biofilms, as compared to the surrounding seawater. Screening results indicated a generally low abundance but a highly diverse and variable nature of harmful pathogens on plastics, largely governed by the type of polymer. Harmful species, such as those from *Vibrio*, *Acinetobacter*, and *Pseudomonas*, were found sporadically recruited. In microcosms, dominating taxa of potential pathogen concern, such as *Pseudomonas* and *Pseudoalteromonas*, can peak and decrease within a potentially degrading biofilm as early as day 7 to 14. When further subjected to antifoulant treatment as an imposed environmental stress over time, *Psuedoalteromonas* can show sharp increases in abundance after three days of exposure but quickly diminished by 14 days in favor of genera such as *Acinetobacter*, *Pseudomonas*, and *Staphylococcus*. From our incubation studies, environmental microplastics have also been shown to concentrate and exacerbate average waterborne exposure to pathogens and ARGs, situationally up to sevenfold or more, per unit volume. These results suggest that, in the context of mariculture, plastisphere-biofilms may promote the early selection, enrichment, and spread of potent, harmful microbial assemblages in the aquatic environment, which could later be exacerbated under mariculture-related chemical and long-term pressures. This study thus contributes to the understanding of microbial succession and associated health risks involved in emerging plastisphere biofilms in light of global plastic pollution.

P3. Quantitative Assessment of Vancomycin Penetration Through Staphylococcal Biofilms

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Objectives: The semi-quantitative biofilm penetration assay combining antibiotic disk diffusion and colony biofilm modelling is inapplicable for antimicrobial agents with a large molecular size such as vancomycin. We modified the diffusion-based biofilm penetration assay and aim to accurately assess vancomycin penetration through staphylococcal biofilms.
Methods: Biofilm-producing *Staphylococcus* laboratory reference strains and clinical isolates were used for this study. Absorbent pads (25 mm diameter, 1 mm thickness) were used as reservoirs for the vancomycin solution, replacing Muller-Hinton agar plates. Overnight incubation allowed antibiotic to penetrate through colony biofilms from the absorbent pad and reach a blank drug disk placed on the top of biofilm. Ultra-Performance Liquid Chromatography was used to determine the concentration of vancomycin in the drug disk. The biofilm penetration ratio was calculated by comparing concentrations of active antibiotics in the disk with or without biofilm barriers.
Results: Vancomycin had a poor penetration through biofilms formed by staphylococcal laboratory reference strains and clinical isolates, with penetration ratios ranging from 13.1 ± 0.5% to 20.9 ± 2.4 %.
Conclusions: The modified method can be used to quantitatively assess biofilm penetration of antimicrobials with a large molecular size, including antimicrobial polymers or peptide mimics that can only be qualitatively examined previously.

P4. Decoding the Multifunctional Genome of the Pf4 Filamentous Prophage: From Phage Genome Editing to Host Interactions

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Pf filamentous prophages are ubiquitous in *Pseudomonas aeruginosa*. Upon activation within biofilms, they serve as crucial mediators of cross-kingdom interactions involving the phage itself, its bacterial host, and eukaryotic organisms. The most studied Pf4 prophage in PAO1 harbors a 12,416 bp ssDNA genome encoding only 20 genes, representing one of the simplest known life forms. Here, we elucidate the biological functions of these genes. A retron-like locus near the attachment site, comprising the ncRNA PhrD, reverse transcriptase PfrT, and putative endonuclease PfrE, functions as a tripartite toxin-antitoxin system. Deletion of any component impairs Pf4 excision in the biofilm microenvironment. Furthermore, PhrD and PfrT collaborate to edit the Pf4 genome, generating superinfective variants that propagate rapidly within biofilms (Guo et al., 2024, *Cell Reports*). Adjacent to this locus, genes governing the lysogeny-lysis switch include the repressor *pf4r* and excisionase *xisF4*, which maintain lysogeny and promote Pf4 excision, respectively (Li et al., 2019, *Molecular Microbiology*). The core genomic region, comprising 13 genes, directs phage replication and assembly. RepG4 is essential for Pf4 replication (Gu et al., 2025, *Nature Communications*), while PflM contributes to lysogeny maintenance. The function of the downstream gene *PA0719* remains unknown. The following *gV* encodes the ssDNA-binding protein, involved in phage assembly. The five capsid genes, encoding one major capsid *gVIII*, and four minor capsids *gVII* (*pfsE*), *gIX*, *gIII*, *gVI*, form the virion structure, with *gVII* and *gIII* also mediating superinfection exclusion by interfering with type IV pilus function (Wang et al., 2022, *Environmental Microbiology*), and they also confer phage defense activity. PftO4 (Zot) facilitates phage release, and the co-transcribed 102 bp gene *pftP4* exhibits *P. aeruginosa*-specific killing activity (Weng et al., 2025, *Viruses*). Rep4 and IntF4 encode the replication initiator and integrase, respectively. Finally, a downstream type II toxin-antitoxin system, PfiAT, suppresses Pf4 prophage activation (Li et al., 2020, *Microbial Biotechnology*; Chen et al., *Science Advances*, under revision). Collectively, our findings demonstrate that the minimal Pf4 prophage genome encodes a sophisticated repertoire of functional modules regulating its lifecycle, genome plasticity, and host interactions.

P5. Beyond PIA: Spx Enhances *S. aureus* Biofilm Formation via Fibronectin-Binding Protein-Dependent Adhesion

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Staphylococcus aureus biofilms are major contributors to chronic device-associated infections. This study identifies the transcriptional regulator Spx as a central positive determinant of biofilm formation under both static and physiologically relevant fluid shear conditions. Genetic knockdown of *spx* severely impaired biofilm biomass and stability, while its overexpression enhanced the formation of dense, shear-resistant biofilms. Integrated transcriptomic and proteomic analyses revealed that Spx strongly upregulates the expression of fibronectin-binding proteins FnBPA and FnBPB. Functional assays confirmed that altered *spx* expression directly modulates bacterial adhesion to abiotic surfaces and to human nasal epithelial cells in an FnBPA/B-dependent manner. Notably, while *spx* knockdown increased polysaccharide intercellular adhesin (PIA) production, biofilm integrity was compromised, indicating that Spx-mediated biofilm robustness primarily depends on proteinaceous adhesins rather than PIA. Crucially, in a rabbit subcutaneous catheter model, *spx* knockdown resulted in scant, underdeveloped biofilms, whereas control and *spx*-overexpressing strains formed thick, host cell-integrated biofilms. These findings establish Spx as a pivotal regulator of *S. aureus* USA300 biofilm formation and device-related pathogenesis, controlling FnBPA and FnBPB adhesins beyond PIA.

P6. Mechanism of Mammalian Body-Heat Sensing by a Bacterial Thermosensory Diguanylate Cyclase

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In *Pseudomonas aeruginosa*, the intracellular second messenger cyclic diguanylate (c-di-GMP) regulates extracellular polysaccharide production, motility, and virulence. Our group has discovered the thermosensory diguanylate cyclase (TdcA), which synthesizes c-di-GMP at mammalian body temperature, modulating *P. aeruginosa* virulence traits. Temperature-sensing is mediated by a thermosensitive PER-ARNT-SIM (thermoPAS) domain that regulates an adjacent GGDEF domain for c-di-GMP synthesis; however, the mechanism of thermal sensing remains unknown. Here, we used integrative structural biology approaches, synthetic enzyme design, genetic analyses, and biochemical assays to define features essential for TdcA's temperature-dependent activity. Hydrogen-deuterium exchange mass spectrometry identified five heat-labile regions in TdcA: an N-terminal amphipathic region, the F and Jα-helices, and the G and Ib strands of thermoPAS. Cryo-electron microscopy and AlphaFold3 modelling were used to produce a preliminary structure of a 74 kDa TdcA dimer with local resolution as low as 3.74 Ångstroms. Bioinformatics identified the amphipathic N-terminus as a signature of TdcA orthologs. Mutagenesis of this region abolished temperature sensitivity *in vitro* and in vivo. Subcellular fractionation and immunoblotting revealed that TdcA is localized to the inner membrane by its N-terminus. Mutagenesis showed that regions beyond the PAS core, including the N-terminus and linker, are essential for thermal activation. Linker mutations locked the enzyme "on" or "off." A synthetic β-galactosidase bifurcated with thermoPAS displayed temperature-dependent activity when thermoPAS remained intact and properly positioned. Cross-linking MS supported temperature-dependent rearrangement of the N-terminal amphipathic region. Taken together, we propose that TdcA functions as a membrane-coupled thermosensory device. Temperature-dependent structural changes are transmitted through an axis originating in a membrane-embedded, N-terminal amphipathic helix, through the PAS domain and linker, and terminating in the GGDEF domain to reorient the catalytic site of a dimer. These findings reveal modular accessory elements to the canonical PAS domain that are fundamental to thermal sensation in TdcA.

P7. Exploring a New Resistance-Breaking Strategy Against Carbapenem-Resistant Enterobacteriaceae

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Carbapenem-resistant Enterobacteriaceae (CRE) infections have limited treatment options and often require combination therapy, prolonged hospitalization, and result in high morbidity and mortality. These challenges are jeopardized when CRE form biofilms, which further reduce antibiotic penetration. To address this, this study will investigate two key reasons for carbapenem resistance: the production of carbapenemases and efflux pump overexpression and their relation to biofilm formation. In collaboration with Neolixir Ltd. (Lixa), novel resistance breaking molecules will be explored to disrupt biofilms and restore antibiotic effectiveness in both biofilm and planktonic states. Five model Enterobacteriaceae strains (*Klebsiella pneumoniae* ATCC BAA-1705 and ATCC BAA-2342, *Escherichia coli* ATCC BAA-2340, *Enterobacter hormaechei* subsp. xiangfangensis ATCC BAA-2341, and *E. asburiae*) were selected. All strains, except *E. ausburiae* (an environmental isolate), were isolated from clinical specimens. Minimum inhibitory concentrations (MICs) were determined by broth dilution following CLSI guidelines. The next steps will be developing biofilm models using the MBEC device under varying nutrient conditions, including a surrogate Urinary tract infection media. Later, carbapenemase and efflux pump activity will be quantified before and after treatment with new resistance-breaking compounds. Expression analysis as well as high-resolution confocal live-cell imaging will be conducted to monitor real-time changes in the biofilm. To date, MIC testing showed that four clinical isolates were resistant to meropenem (≥4 µg/mL), ertapenem (≥2 µg/mL) and amikacin (≥16 µg/mL), with no colistin resistance detected (CLSI 2025). Carbapenemase inactivation assay confirmed carbapenemase production in all four CRE isolates. The Congo red assay for biofilm screening qualitatively indicated that all isolates were capable of forming biofilms, while quantitative assays showed that *K. pneumoniae* isolates produced significantly greater biofilm after 48 hours in Luria–Bertani broth. Next steps include optimizing biofilm models under varied nutrient conditions, including a surrogate urinary tract infection medium, followed by efflux system analysis, and evaluation of Lixa compounds to potentiate antibiotic activity.

P8. Human Pathogen Quorum Sensing Molecules Regulating Biofilm Formations and Host Programmed Cell Death

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Ferroptosis is a type of iron- and lipid peroxidation-dependent programmed cell death that is involved in various diseases. Some pathogens manipulate host ferroptosis for pathogenesis; however, the potential mechanisms of action remain unclear. *Pseudomonas aeruginosa* is an opportunistic pathogen that relies on iron for its virulence, biofilm formation, and survival. Here, we report that *P. aeruginosa* employs the quorum-sensing metabolite, *Pseudomonas* quinolone signal (PQS), to induce ferroptosis in macrophages through a carnosine-N-methyltransferase (CNMT)-transferrin receptor 1 (TFR1) methylation pathway. Specifically, PQS promotes iron-dependent lipid peroxidation to induce ferroptosis in macrophages. Using high-resolution mass spectrometry–based cellular thermal shift assay (MS-CETSA)/thermal proteome profiling, we identify CNMT as the direct intracellular receptor of PQS in macrophages. Mechanistically, PQS binding increases the histidine methyltransferase (His MTase) activity of CNMT, catalysing methylation of TFR1 at His35. This methylation increases TFR1 protein production, resulting in amplified iron acquisition for ferroptosis. Crucially, the PQS–CNMT–TFR1 axis is distinct from canonical bacterial pathogens that exploit host cell death pathways, revealing the unique strategy of *P. aeruginosa* to exploit host epigenetic machinery.

P9. Plasma-Activated Water is a Potent Agent Against Biofilms of *Staphylococcus aureus* Small Colony Variants

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Some bacteria can form small colony variants (SCVs) as an adaptation mechanism to environmental stress. SCVs are ≤10% the size of the wild type (WT) strain, exhibiting slow growth, altered metabolism, reduced virulence, and are typically associated with increased antibiotic-resistance and biofilms in chronic wounds. Even with current therapies, these communities often persist, and it is unclear whether new treatments can eradicate both WT and SCV populations. Therefore, we evaluated the anti-biofilm efficacy of plasma-activated water (PAW); an innovative solution rich in reactive oxygen and nitrogen species generated by physical plasma treatment of water, on WT and SCV *S. aureus* biofilms. We used two well characterised *S. aureus* SCV strains (JB-1, WCH-SK2 SCV) and their respective WT strains (ATCC-6850, WCH-SK2 WT). PAW significantly reduced the viability of all strains in a concentration-dependent manner, with a ≥4.7 log reduction after 2.5 min with 90% PAW (below detection limit). Phenotype did not affect planktonic colony forming unit reduction for JB-1 or ATCC-6850, but there was a significantly stronger reduction (up to 4.5 logs; p=0.0091) for WCH-SK2 SCV compared to the WT at 25% PAW exposure. For 2-day biofilms, viability of ATCC-6850, JB-1, WCH-SK2 WT and SCV was significantly reduced by 3.7 log (p=0.0064), 4.4 log (p=0.0021), 4.1 log (p=0.0101) and 3.5 log (p=0.0006), respectively. In 7-day biofilms, viability of ATCC-6850, JB-1, WCH-SK2 WT and SCV was reduced by 3.2 log (p=0.0041), 3.3 log (p=0.0115), 4.2 log (p=0.0432) and 3.6 log (p=0.0252), respectively. The WCH-SK2 WT and SCV biofilms were more 4–5-fold more susceptible to amoxicillin and linezolid after PAW exposure (dose-response analysis following 2.5 min PAW treatment; p≤0.038). Overall, PAW was equally potent at killing *S. aureus* SCVs and their WT strains, suggesting that SCVs may not be more adaptable to survive PAW treatment in chronic wounds.

P10. Do All Dog Ear Cleaners Work Alike? A Comparative Study On Antibiofilm Effects in Canine Otitis Externa Management

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Biofilm production by canine otitis externa (COE) pathogens is one of the important factors for resistance development against existing antimicrobials. Alteration in antimicrobial susceptibility and recurrence of COE due to biofilm formation are commonly reported problems in pet clinics but has received less attention compared to human medicine. Dog ear cleaners are recommended in most clinical guidelines to treat the cases of COE and especially it is important in context of antimicrobial stewardship. Ear cleaners play important role in COE treatments by reducing debris and antimicrobial load, disrupting biofilms and enhancing topical antimicrobials' efficacy. The aim of this study was to compare the antibiofilm activity of commonly used canine ear cleaners, including compounding pharmacy formulations, Tris-EDTA, and N-acetylcysteine, with the commercially approved product Otoflush against commonly associated COE pathogens *i.e., Staphylococcus pseudintermedius, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Streptococcus canis* and *Malassezia pachydermatis*. Biofilm production of each isolate was determined by Congo-red agar and microtiter plate assay methods and ten best biofilm-producing isolates from each species were selected to determine the minimum biofilm eradication concentration (MBEC). Biofilms were grown on pegs of the MBEC plates and were challenged by two-fold serially diluted adjuvants. Out of these three ear cleaners, Otoflush® had good antibiofilm activity in moderate to high dilution. N-acetylcysteine showed antibiofilm activity at its highest concentration only for few isolates but is contraindicated in cases of tympanic membrane damage. Tris-EDTA had no antibiofilm activity at its highest concentration. Based on *in vitro* results Otoflush® has intrinsic antimicrobial and antibiofilm activity and is a promising ear cleaner to be used to treat COE cases.

P11. Comparative Analysis of Condition - Dependent Biofilm Formation in *E. coli* and *Salmonella*

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Bacterial biofilms constitute a major clinical and industrial challenge, conferring enhanced tolerance to antimicrobial agents and significantly contributing to the global burden of antimicrobial resistance. Biofilm formation is a key survival strategy in many bacterial pathogens including notorious food pathogens, *Escherichia coli* and *Salmonella*. However, it is highly dynamic and strongly influenced by environmental factors such as temperature, nutrient availability and surface condition among others. In this study, we investigated condition-dependent biofilm formation in mono- and dual-species biofilms of *E. coli* strains (ATCC25922, BMH8117A, JM101 and MB14) and *Salmonella* strains (*S. enteritidis* 11RX-1, *S. enteritidis* 11RX-2, *S. enterica* typhimurium M338, and *S. enterica* 700720) using static MBEC™ Model. Biofilm biomass, metabolic activity, and viability were assessed using crystal violet assay, resazurin and plate count methods to capture complementary aspects of biofilm development across different growth conditions. MBEC peg lids were coated with bovine serum albumin (BSA), and collagen to evaluate the influence of host factors in biofilm formation. Data analysis was achieved using two-way ANOVA in GraphPad Prism. Robust biofilm formation was observed in nutrient-rich TSB media in *E. coli* single and co-cultures, while optimization in nutrient-poor media revealed differential biofilm responses, particularly for *Salmonella* strains. Additionally, biofilm development differed between coated and uncoated surfaces. Most *E. coli* and *Salmonella* strains produced lower biomass in coated plates compared to the uncoated. It was further observed that BSA did not enhance initial attachment of cells to promote biofilm formation. These findings demonstrate that biofilm formation is highly context-specific and underscore the importance of environmental and interspecies factors in shaping biofilm behavior. Incorporating condition-dependent and multispecies perspectives is critical for developing more effective antibiofilm interventions. Future work will focus on using fluorophore plasmids to understand the dynamic interactions in co-cultures and confocal imaging to understand composition and changes in biofilm formation.

P12. Unravelling Novel Resistance Genes, Virulence Factors, and Therapeutic Targets in Multidrug-Resistant *Pseudomonas aeruginosa* Isolates Recovered from Keratitis Patients

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Background: The opportunistic pathogen *Pseudomonas aeruginosa* has become a major clinical and public health concern due to its increasing rates of multidrug (MDR) and pandrug resistance. Whilst ocular infection with *P. aeruginosa* can result in blindness, the effect of increased antibiotic resistance is highlighted by an outbreak of extensively drug-resistant (XDR) *P. aeruginosa* ocular infections in USA in 2023 that resulted in four deaths, four eye removals, and severe vision loss in another 14 people. The full complexity of antibiotic resistance and virulence in ocular isolates in not known. Hence, this study aimed to identify the resistance genes and virulence factors produced by *P. aeruginosa* isolated from ocular infections around the world.
Methods: Phenotypic characterization, including the determination of minimum inhibitory concentrations (MICs) and the assessment of biofilm-forming ability of 12 *P. aeruginosa* isolates obtained from ocular infections, was performed using the broth microdilution method and crystal violet assay, respectively. Whole-genome sequencing was carried out using the Illumina sequencing platform and annotated with Prokka v1.14.6. AMR genes were identified using AMRFinderPlus v4.0.23 and the Comprehensive Antibiotic Resistance Database (CARD) v3.3.4. The presence of acquired resistance genes and virulence factors was further analyzed using ResFinder v4.7.2 and the Virulence Factor Database (VFDB), respectively. Moreover, roary v3.13.0 was used for pangenome analysis, while Snippy v4.6.0 was employed for whole-genome variant analysis.
Results: A third of the ocular isolates were MDR. However, all isolates were sensitive to polymyxin B, and two thirds were sensitive to gentamicin, tobramycin, and levofloxacin. Two thirds of the isolates were strong biofilm producers, with all MDR isolates being strong biofilm producers (Table 1). The AMR genes *catB7*, *fosA*, and *aph(3')-IIb* were detected in nearly all isolates. Interestingly, MDR *P. aeruginosa* isolates uniquely carried aminoglycoside resistance genes (*aac(3)-IId*, *aac(6')-Ib4*, *aadA1*, *aadA11*, *aph(3')-I(H957)*, *aph(6)-Id*M, *rmtF2*, *rmtB4*, *rmtD*); β-lactam resistance genes (*blaGES-9*, *blaLCR-1*, *blaOXA-10*, *blaOXA-395*, *blaOXA-488*, *blaPAU-1*, *blaPDC-19a*, *blaPDC-36*, *blaTEM-1*, *blaVIM-80*); fluoroquinolone resistance genes (*qnrVC1* and *gyrA*); tetracycline resistance gene (*tet(G)*); macrolide resistance genes (*ere(A)*, *mph(A)*, *mph(E)*, *msr(E)*); and other resistance genes (*dfrA5*, *floR*, *floR2*, *qacE*, *qacEdelta1*, *tmexC*, *tmexD2*, *toprJ1*) (Table 2). Many ocular isolates obtained from India were MDR and harbored a greater number of resistance and virulence genes compared to the Australian isolates. Interestingly, MDR isolates were *exoU*-positive (that encodes for a potent phospholipase), whereas non-MDR isolates were

exoS-positive (that encodes for a dual function Rho GTPase and an ADP-ribosyltransferase protein)(Table 3).
More non-synonymous variations were detected in MDR *P. aeruginosa* strong biofilm–producing strains (total SNPs: 205,950) compared to strong (total SNPs: 151,096) and weak (total SNPs: 105,212) biofilm–producing non-MDR *P. aeruginosa* strains (Table 4). MDR *P. aeruginosa* isolates exhibited a relatively larger pangenome compared to non-MDR isolates, and the phylogenetic analysis showed that the MDR isolates clustered together (Figure 1).
Conclusions: Despite the presence of MDR strains, most keratitis *P. aeruginosa* isolates remained susceptible to key antibiotics, whereas MDR isolates possessed additional resistance and virulence genes. Indian ocular isolates were predominantly MDR and harbored more resistance and virulence genes than Australian isolates. Therefore, targeting the unique genes associated with MDR *P. aeruginosa*, together with the potent virulence factor *exoU*, may provide an effective strategy to mitigate the spread of MDR *P. aeruginosa* strains.

P13. A Noncanonical Intrinsic Terminator in the Toxin–Antitoxin Operon Promotes the Transmission of Conjugative Antibiotic Resistance Plasmids

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Conjugative plasmids, major vehicles for the spread of antibiotic resistance genes, often contain multiple toxin–antitoxin (TA) systems. However, the physiological functions of TA systems remain obscure. By studying two TA families commonly found on colistin-resistant IncI2 *mcr-1*-bearing plasmids, we discovered that the HicAB TA, rather than the StbDE TA, acts as a crucial addiction module to increase horizontal plasmid–plasmid competition. In contrast to the canonical type II TA systems in which the TA genes are cotranscribed and/or the antitoxin gene has an additional promoter to allow for an increased antitoxin/toxin ratio, the HicAB TA system with the toxin gene preceding the antitoxin gene employs internal transcription termination to allow for a higher toxin production. This intrinsic terminator, featuring a G/C-rich hairpin with a UUU tract, lies upstream of the antitoxin gene, introducing a unique mechanism for enhancing toxin/antitoxin ratio output. Critically, the *hicAB* TA significantly contributes to plasmid competition and plasmid persistence in the absence of antibiotic selection, and deleting this intrinsic terminator alone diminishes this function. These findings align with the observed high occurrence of *hicAB* in IncI2 plasmids and the persistence of these plasmids after banning colistin as a feed additive. This study reveals how reprogramming the regulatory circuits of TA operons impacts plasmid occupancy in the microbial community and provides critical targets for combating antibiotic resistance.

Keywords: toxin–antitoxin system; intrinsic terminator; *mcr-1*; conjugative plasmid; antibiotic resistance genes

P14. Utilising Lightning Within the Food Industry: Plasma-Activated Liquids to Combat Foodborne Pathogens

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Introduction: Foodborne pathogens pose a significant public health challenge in Australia, resulting in approximately 48,000 hospital admissions annually and an estimated economic impact of $3 billion. The rising threat of antimicrobial resistance reduces the efficacy of conventional food sanitisers, increasing the risk of contamination. Plasma-activated media (PAM) are innovative sanitisers generated by exposing water, saline, or buffer solutions to ionised gases, producing antimicrobial reactive oxygen and nitrogen species. These sanitisers have substantial potential for the food sector, as they contain minimal to no industrial chemicals and can reduce the environmental impact of food sanitisation.
Hypothesis: PAM generated in water, saline, and buffer solutions exhibits antimicrobial and antibiofilm efficacy against foodborne pathogens. This was evaluated using planktonic suspensions and biofilms of *Listeria monocytogenes* and *Escherichia coli*.
Methods: The effects of different base media and generation times were analysed over 7 days by monitoring physicochemical properties of each PAM, including pH, conductivity, and oxidative-reductive potential. The concentrations of reactive oxygen and nitrogen

species were quantified using UV-VIS spectrophotometry. To evaluate antimicrobial efficacy, time-kill experiments were conducted with PAM generated for 5, 10, 20 and 40 minutes against suspensions of *Listeria monocytogenes* and *Escherichia coli*, each standardised to $1x10^8$ CFU/ml. Additionally, antibiofilm activity was assessed against 48-hour biofilms using the AlamarBlue viability assay and CFU extraction.
Results: The base medium and generation time significantly influenced pH, conductivity, ORP, and ion concentrations in each PAM. Time-kill curve experiments demonstrated a reduction of up to 5.8 to 6.0 log in planktonic bacteria within 20 seconds when using PAM generated for 40 minutes, and up to a 99% reduction in biofilm viability across all assessed strains.
Conclusion: The tested PAMs show promising antimicrobial and antibiofilm properties against foodborne pathogens. Future research could support the translation of these formulations into practical food industry applications for food safety.

P15. Deciphering the Composition and Roles of the Biofilm Matrix in the Great Adapter *Burkholderia pseudomallei*

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Burkholderia pseudomallei (*Bp*) forms robust biofilms and persists in the environment. *Bp* is also resistant to many antimicrobials and is the causative agent of melioidosis, a severe infectious disease with global distribution and high mortality rates. As a sapronotic disease agent, *Bp* can effectively transit from environmental reservoirs to cause infection in a host, but little is known about how it senses and responds to alter the production of the molecules that contribute to biofilm formation during this transition. *Bp* produces a diverse array of surface-associated proteins and polysaccharides, which factor in its adaptability and biofilm production capabilities. Additionally, it produces numerous secondary metabolites that contribute to and alter biofilm growth dynamics. Together, these molecules likely contribute to the ability of *Bp* to produce a biofilm and to readily adapt to changing environmental cues. The aim of these studies is to understand how this complex repertoire of extracellular matrix components is altered in response to environmental cues and the various roles that they possess. To study these molecules, we used a variety of genetic approaches to disrupt production of matrix components and the signaling systems that control their production, while concurrently altering the environmental conditions. Targeted disruption of c-di-GMP messaging revealed phenotypic impacts on biofilm formation, colony morphology, motility, and secondary metabolites in response to environmental cues. Deletion analysis of the biosynthetic gene clusters encoding the surface-associated polysaccharides identified specific roles for some components and overlapping functions for others. These approaches have the potential to decode the cryptic expression and roles of these matrix components that contribute to *Bp* biofilm formation. The ability of *Bp* to produce numerous surface-associated proteins and polysaccharides contributes to its plasticity and survival in diverse environments and niches.

P16. Multimodal Computational Identification of MvfR Inhibitors Targeting Quorum Sensing in Multi-Drug-Resistant *Pseudomonas aeruginosa*: Insights from AI Modelling, Molecular Docking, ADME, Molecular Dynamics Simulations, and Quantum Chemical Calculations

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Pseudomonas aeruginosa is a major global health concern due to its multidrug resistance (MDR), necessitating the urgent development of novel therapeutic strategies. Understanding the molecular basis of resistance in clinical isolates is critical for designing next-generation antimicrobials.
This study analysed recent clinical isolates of *P. aeruginosa* obtained from the NCBI for their resistance gene and virulence factor profiles. Among the virulence-associated targets, MvfR, a key transcriptional regulator of quorum sensing and biofilm formation, was prioritized based on its functional relevance. AI modelling of MvfR identified from the genome analysis was performed, followed by molecular docking against a library of compounds, phylogenetic comparisons to compare with previously identified one, ADMET-profiling, 500ns molecular dynamics (MD) simulations, free binding energy, and Density Functional Theory (DFT).
Genes critical for antimicrobial resistance, drug targeting, and virulence factors were identified across multiple databases. The antimicrobial resistance genes and receptors

revealed key resistance mechanisms, including antibiotic-inactivating enzymes, efflux pumps, quorum sensing, and alterations in cell wall charge or permeability. Notably, (S)-1-(2-(difluoromethyl)-1H-benzo[d]imidazol-5-yl)-3-(2-hydroxy-2-(pyridin-4-yl)ethyl)urea exhibited the highest docking scores against MvfR. DFT and MD simulations over 500 ns demonstrated stability of the top ligands, supported by favourable molecular stability parameters such as RMSD, SASA, RMSF, and Rg plots. Furthermore, the top-ranking ligands satisfied Lipinski's rule of five, suggesting favourable drug-like properties.
This study provides an integrated computational characterization of MvfR in recent *P. aeruginosa* isolates and identifies genetic variations that may influence disease manifestation.

P17. Disruption of *P. aeruginosa* and *S. aureus* Dual Species Biofilms with Antimicrobial Peptide (LL-37) and Ciprofloxacin

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Pseudomonas aeruginosa and *Staphylococcus aureus* frequently form mixed-species biofilms during infection. Antimicrobial peptides (AMPs), including the human cathelicidin LL-37, may inhibit or disrupt biofilms alone or with antibiotics. This study assessed LL-37 and ciprofloxacin, individually and combined, for their ability to disrupt pre-formed single and mixed biofilms. Methods: *P. aeruginosa* PA15 (urine isolate) and *S. aureus* 34654 and 30616 (wound isolates) were grown overnight in tryptic soy broth (TSB), diluted to OD600 0.1, and inoculated individually or 1:1 into 96-well plates. After 24 h at 37˚C, established biofilms were left untreated or exposed to LL-37, ciprofloxacin, or both for 24 h. Biofilms were disrupted by vigorous scrubbing, and viable bacteria were quantified on differential media. Results: In single-species biofilms, 8×MIC ciprofloxacin reduced PA15 (6.8±0.5 to 3.5±0.6 log10 CFU/ml), SA30616 (7.7±0.14 to 6.6±0), and SA34654 (7.3±1.1 to 6.5±0). LL-37 at 2×MIC had no effect on PA15 or SA30616 but reduced SA34654 (7.3±1.1 to 6.0±0). The ciprofloxacin + LL-37 combination further decreased PA15 (to 1.7±2.4) and SA34654 (to 5.5±0.7), with little additional effect on SA30616. In mixed biofilms, PA15 suppressed SA30616 (7.7±0.14 to 5.0±1.9) while increasing its own numbers (6.8±0.5 to 7.6±0.9). With SA34654, PA15 was unchanged (6.7±1.49) and SA34654 decreased slightly (7.3±1.1 to 6.1±3.7). In mixed biofilms, 8×MIC ciprofloxacin reduced PA15 to 3.2 log10 CFU/ml, eradicated SA30616, and reduced SA34654 to 2±0. LL-37 (2×MIC) reduced PA15 to 6.6±0.9 with SA30616 and 3.7±2.9 with SA34654; both S. aureus strains reduced to ~3.0 log10 CFU/ml. The combined agents further reduced PA15 (4.0±0.07 with SA30616; 2.0±2.2 with SA34654) and eliminated SA30616 while lowering SA34654 to 2.5±0.6. Conclusions: In mixed biofilms, PA15 remained similarly susceptible to single agents but showed slightly greater tolerance to the combination. Both *S. aureus* strains became more susceptible to ciprofloxacin, LL-37, and their combination when grown with *P. aeruginosa*.

P18. Formidable Anti-biofilm and Anti-Adhesion Effects of Human Lactoferrin against *Pseudomonas aeruginosa*

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Introduction: Biofilm formation is one method used by *Pseudomonas aeruginosa*, a common cause of keratitis, to resist the effects of antibiotics and host defence systems. Lactoferrin, a natural iron-binding protein found in tears, possesses antimicrobial properties that may help combat *P. aeruginosa* infections by preventing and disrupting biofilm formation.
Objectives: This study examined how human lactoferrin affects *P. aeruginosa* adhesion, biofilm formation, and entry into corneal cells.
Methods: Human lactoferrin antimicrobial, anti-biofilm, and anti-invasion effects against six *P. aeruginosa* strains were measured using minimum inhibitory concentration microdilution assays, crystal violet biofilm inhibition and degradation assays, and viable colony counts. Its ability to protect human corneal epithelial cells (HCE-T) from bacterial invasion was tested using a gentamicin protection assay, while effects on bacterial motility were measured with a twitching assay. Data were analysed using unpaired t-tests with significance set at $p \leq 0.05$.
Results: Lactoferrin showed strong inhibition and dispersal activity against six strong biofilm-forming *P. aeruginosa* strains (PA008, PA016, PA216, PA225, PA232, and ATCC19660). On average, lactoferrin inhibited biofilm formation by ≥ 80%, with 2 mg/mL causing the greatest reduction in biofilm biomass and viable cells. Human lactoferrin degraded 91% biofilm formed by ATCC 19660, and 61% of the biofilm formed by PA008 was

less degraded. Lactoferrin significantly reduced bacterial attachment and invasion into human corneal epithelial cells. More than 70 % of the invasion efficiency of PA216 and PA225 was decreased
Conclusions: Overall, these findings demonstrate that human lactoferrin inhibits *P. aeruginosa* biofilm development, disrupts established biofilms, and limits bacterial adhesion and invasion of corneal epithelial cells. This underscores its action as a natural antimicrobial and may be one reason why the ocular surface is paucimicrobial.

Keywords: Lactoferrin, *Pseudomonas aeruginosa*, biofilm formation, biofilm degradation, cell adhesion, invasion

P19. Nanostructured Driveline Surfaces to Combat Device-Associated Infections and Its Translational Potential

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Patients with end-stage heart failure often depend on ventricular assist devices (VADs) for survival, but biofilm-related driveline infections remain a significant clinical challenge. Nanostructured surfaces have recently emerged as a promising strategy to prevent biofilm formation on medical devices. Inspired by the bactericidal architecture of dragonfly wings, these nanoscale features mechanically disrupt bacterial membranes through sharp, high-aspect-ratio structures. In this study, we engineered anti-infective driveline surfaces using silicon-based, vertically aligned nanowire arrays fabricated through advanced nanomanufacturing techniques. Key nanowire design parameters including height (2 µm), pitch (500 nm), and tip diameter (50 nm) were optimized to enhance bactericidal performance against pathogens commonly responsible for VAD driveline infections. To expand their applicability to curved or flexible device components, the rigid silicon nanowires were successfully transferred onto compliant polymeric substrates. Antimicrobial activity was assessed using colony-forming unit enumeration, scanning electron microscopy (SEM), and focused ion beam-SEM (FIB-SEM). The optimized nanowires exhibited strong bactericidal effects against multiple clinically relevant microorganisms. SEM and FIB-SEM analyses revealed substantial membrane deformation and rupture in bacteria attached to the nanostructured surfaces. Furthermore, the nanowire arrays were successfully replicated onto flexible polymers including PDMS, polystyrene, and polyurethane materials routinely used in medical device manufacturing. Overall, this work presents a translational, antibiotic-free strategy to combat biofilm-associated infections on VAD drivelines and demonstrates the broader potential of nanostructured surfaces to reduce bacterial contamination in diverse biomedical applications.

P20. Phage-Driven Microbiome Shifts: Thermal Stress, Biofilms, and the Prophage Weapon

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Biofilms are critical for microbial colonization and competition in coral holobionts, with prophage-host interactions emerging as key regulators of microbiome dynamics under environmental stress [1,2]. This study aimed to elucidate how temperate phages mediate colonization competition between coral pathogens and commensal bacteria gathering as biofilms in coral holobiont. We isolated culturable bacteria from the gastric cavity of healthy *Galaxea fascicularis* and found that the non-toxigenic commensal *Vibrio alginolyticus* (Va43009) exhibits superior colonization capacity compared to the pathogen *Vibrio coralliilyticus* (Vc43001). However, Vc43001 outcompetes Va43009 under thermal stress by triggering lytic induction of the P2-like prophage GfP2 in Va43009. Mechanistically, Vc43001-encoded LodAB produces hydrogen peroxide, which activates the SOS response and prophage lysis in commensal bacteria. LodAB-dependent prophage induction also enables Vc43001 to suppress other coral symbionts (e.g., *Endozoicomonas* sp.). These findings reveal that pathogens exploit prophage induction as a competitive strategy to colonize corals, highlighting the pivotal role of phage-bacteria interactions in shaping coral microbiome stability and pathogen invasion under environmental change.

References
[1] Rosenberg E, Zilber-Rosenberg I. mBio 2016;7:e01395.
[2] van Oppen MJH, Blackall LL. Nat Rev Microbiol 2019;17:557-567.
Wang W, et al. Nat Ecol Evol 2022;6:1245-1255.

P21. Antibiofilm Efficacy of Phage Cocktails and their Combination with Antibiotics Against *Pseudomonas aeruginosa*

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Purposes: *Pseudomonas aeruginosa* is a common antibiotic-resistant pathogen, posing a significant public health threat worldwide. *P. aeruginosa* produces biofilms during infections, which play a critical role in enhancing antibiotic resistance and its pathogenicity. Bacteriophage therapy is emerging as an option for treating multidrug-resistant *P. aeruginosa* and biofilms. This study aimed to assess the antibiofilm activity of novel phage cocktails alone and in combination with antibiotics, against *P. aeruginosa* biofilms. Method: Two *P. aeruginosa* isolates and six phages were included in this study. Spot tests and time-kill assays were used for phage sensitivity. Thermal and pH stability, host range, efficiency of plating, phage adsorption and one step growth curve were used for phage characterization. Phage morphology was examined using TEM. Phage cocktails alone and in combination with antibiotics were applied against 24 hours and 48 hours grown *P. aeruginosa* biofilms. Their effects on biofilms were measured by crystal violet staining assay and confocal microscopy. Result: All selected phages produced broad zones of inhibition against both *P. aeruginosa* strains. EOP assay showed medium to high productivity across both *P. aeruginosa*. Over 95% of phage particles adsorbed within 5 - 15 minutes. Phage DiSu5 exhibited the highest average burst size of 42 with a latent period of 13 minutes. Among the combinations (33 different phage cocktails), the cocktail containing DiSu1, DiSu4 and DiSu5 was the most effective in reducing bacterial growth and biofilm of both strains. Phage cocktail treatment at an MOI of 1 significantly reduced preformed biofilms of both strains. The cocktail combined with ciprofloxacin produced the best effects. Confocal microscopy further confirmed a markedly increased proportion of dead bacterial cells in treated biofilms in three-dimensional analyses. Conclusion: These findings highlight the therapeutic potential of tailored phage cocktails, particularly when combined with antibiotics for targeting biofilm associated *P. aeruginosa* infection.

P22. ESKAPE-ing Antimicrobial Resistance: 213 nm Laser Light to Combat Superbugs

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Hospital-acquired infections (HAIs) are a major threat to public health affecting 170,000 Australians yearly and causing 70% of antimicrobial resistance deaths. New tools to combat HAIs are direly needed, especially HAIs caused by ESKAPE pathogens (*E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* sp.). Ultraviolet (UV) light (<400 nm) is used for surface disinfection; however, toxic side effects limit clinical applications. In contrast, far-UV light (200-230 nm) has been demonstrated as safe and antimicrobial, making it a promising alternative to combat ESKAPE pathogens. We hypothesised that novel 213 nm far-UV laser light is antimicrobial. We aimed to evaluate its efficacy against 6 ESKAPE pathogens in dry and suspended conditions. *E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *E. cloacae* were used to assess laser antimicrobial efficacy. For the dry state, bacteria were spread on Tryptone Soya Agar, dried, and exposed to the laser for 5ns to 300s. After overnight incubation (37˚C), inhibition zones were quantified using ImageJ (n≥3). For the suspension, bacteria were suspended in 0.9% NaCl and exposed to the laser (1s-900s). Bacterial killing was quantified by colony forming unit count (n=3). Statistical significance ($p<0.05$): one-way ANOVA with Tukey's multiple comparison test. The 213 nm laser light was highly effective against all planktonic ESKAPE pathogens in dry conditions, with growth inhibition observed after 1s of exposure. Bacteria in suspension required higher doses of light to achieve disinfection. Significant reductions in bacteria were seen at 1s of treatment ($p<0.01$) and a 2-log reduction ($p<0.0001$) was seen after 900s. Far-UV light showed antimicrobial activity against all ESKAPE pathogens in clinically relevant

timeframes. Our data suggests that 213 nm lasers hold potential as a novel tool to combat HAIs with antimicrobial resistant bacteria.

P23. Synergistic Antibiotic Effects on *Pseudomonas aeruginosa* with Multiple Resistance Mechanisms

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Introduction. *Pseudomonas aeruginosa* has a large armamentarium of resistance mechanisms enabling resistance emergence during therapy against almost all antibiotics in monotherapy, in addition to its ability to form biofilms. Pharmacokinetic/pharmacodynamic (PK/PD) indices link bacterial response to antibiotic exposure, but cannot account for combination therapies. Biofilms are also biologically distinct from planktonic growth and more research is needed to better treat these infections.
Aims. To evaluate dosing regimens of meropenem (MER) and ceftolozane/tazobactam (C/T), alone and combined, against well-characterised *P. aeruginosa* strains with multiple resistance mechanisms in a CDC dynamic *in vitro* biofilm reactor, and quantify planktonic and biofilm growth.
Methods. *P. aeruginosa* strains PAO1ΔADΔmexR and PA14ΔADΔmexR, derived from the wild-type strains PAO1 and PA14, respectively, were used. Both strains had *ampD* knock-out leading to increased β-lactamase production and *mexR* knockout resulting in efflux pump overexpression. MICs were determined in triplicate. Dosing regimens were: MER continuous infusion (6g daily), C/T intermittent infusions (as Zerbaxa 1.5g [1g ceftolozane 0.5g tazobactam] or 3g, 8-hourly [Q8] as 3-h infusions), and both combinations. The study was run for 168h.
Results. MIC_{MER} was 4 mg/L for PAO1ΔADΔmexR and 2 mg/L for PA14ΔADΔmexR. $MIC_{C/T}$ was 2 mg/L for PAO1ΔADΔmexR and 1 mg/L for PA14ΔADΔmexR. For PAO1ΔADΔmexR all monotherapies resulted in regrowth and amplification of MER- and C/T-resistant subpopulations (Figure). The combination regimens were synergistic and suppressed total and resistant bacteria. Conversely, for PA14ΔADΔmexR the monotherapies provided suppression of regrowth and resistance emergence, and thus combination therapies were not pursued. These results were consistent between the planktonic and biofilm growth of each strain.
Discussion. Combination regimens of MER and C/T successfully suppressed resistance emergence against *P. aeruginosa* strain PAO1ΔADΔmexR. The different effects of monotherapies across the strains reflect differences in MIC. Future steps include developing a mechanism-based model that may ultimately be used to optimise dosing regimens.

P24. Defying Gravity: Cold Plasma Technology to Defeat Superbugs in Space Exploration

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In space, altered gravity and weakened immunity due to space radiation increase infection risk, partly due to enhanced biofilm formation. Confined to the hermetic environment of a spacecraft, stressors like microgravity, radiation and restricted antibiotic diversity can enhance biofilm tolerance. As future space missions grow longer, it is unclear whether existing treatments remain effective, therefore space-compatible antibiofilm strategies are urgently needed. Plasma-activated water (PAW), created by treating water with cold plasma to produce reactive species, has shown promise as an antibiotic-free antimicrobial. Therefore, this study tested whether PAW retains its antibiofilm activity after irradiation and whether biofilms grown in simulated microgravity vs terrestrial conditions respond differently to PAW. We evaluated the efficacy of

irradiated PAW against *S. aureus* biofilms grown terrestrially vs in simulated microgravity. Biofilms were grown for 24h using a random positioning machine (ANFF) to simulate microgravity, alongside terrestrial controls. PAW was irradiated with 0.1 and 0.2 Gray using a 6 MV X-ray beam from a linear accelerator (ARPANSA), alongside water and pH controls (HNO_3). Physicochemical properties including pH, conductivity and oxidative-reductive potential (ORP) were tracked over 3 months. Biofilms were treated with irradiated PAW for 5 min, and antibiofilm activity was assessed by colony-forming unit (CFU/mL) counts (n3). Statistical analysis: two-way ANOVA and Šídák's multiple comparison tests ($p<0.05$). PAW showed no statistically significant differences between radiation conditions for pH, conductivity or ORP. Terrestrially-grown biofilms experienced 2-log reductions after HNO_3 treatment ($p<0.05$) and 3-log reductions after PAW ($p<0.0001$), with non-significant differences between radiation doses. Simulated microgravity biofilms were more robust, with 1-log reductions after HNO_3 or PAW treatment ($p<0.05$). Overall, irradiated PAW effectively reduced terrestrial biofilms without changes in physicochemical properties, while microgravity-grown biofilms showed enhanced resilience. This underscores biofilm contamination as a key challenge for long-duration spaceflight, and furthermore supports PAW's potential as a space-compatible antimicrobial.

P25. Copper Nickel Passivation and the Development of Corrosive Microbial Biofilms

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Copper nickel (CuNi) alloys are widely used in maritime vessels and structures due in part to their excellent corrosion resistance in seawater. This resistance to degradation is helped by the formation of surface copper oxide layers which create a barrier between the environment and the base material. There are a variety of methods to enhance this passive layer, the effectiveness of which in turn can be affected by environmental conditions present during the passive layer formation. Previous research has shown that the passivation of CuNi can significantly reduce general corrosion; however, recent work has shown that in the presence of certain microorganisms the passivation process may actually lead to increased corrosion rates.
Marine environments can support the growth and development of complex biofilms, with biofouling being a major concern for many maritime industries. In certain situations, these biofilms can host many microorganisms that are able to cause highly accelerated localised corrosion known as microbially influenced corrosion (MIC). Previous research on passivation and MIC has largely focussed on tests using single strains of bacteria, which is not representative of real-world environments. The current work is assessing the development of passive layers under varying seawater conditions with subsequent testing where these passivated metals are exposed to an MIC test. The MIC test was conducted with a complex microbial community sourced directly from the field to emulate more natural environmental conditions. The presentation will discuss the results of this testing including a focus on 16S rRNA gene sequencing, utilised to determine differences in the community composition of biofilms formed for passive films developed under varying test conditions.

P26. Lactoferrin Acts Synergistically with Flucloxacillin Against *Staphylococcus aureus* Biofilms via a Hydrogel–Niosome Delivery System

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Background: Biofilm formation is one of the primary pathogenic mechanisms of staphylococcal osteomyelitis, contributing significantly to the chronicity and recurrence of the infection. However, there is currently no effective treatment specifically targeting biofilm-associated infections. Lactoferrin, an 80-kDa iron-binding glycoprotein, possesses broad-spectrum antimicrobial and bactericidal activities. The aim of this study is to investigate the efficacy of lactoferrin in combination with flucloxacillin in eradicating staphylococcal biofilms.
Methods: A CDC Biofilm Reactor was utilized to cultivate mature *Staphylococcus aureus* Xen-36 biofilms on implant-grade stainless steel coupons for 3 days. Structurally stable biofilms were obtained through continuous replacement of nutrient-rich media and saline wash. The coupons were then randomly placed into treatment solutions of the control group, lactoferrin group, flucloxacillin group, and flucloxacillin–lactoferrin group for a 3-

day synergy assay. Fresh treatment medium was replaced daily, and bacterial cells from both the culture media and coupons were collected and quantified using serial dilution plating to determine colony-forming units (CFUs). On this basis, a biodegradable Hydrogel-Niosome system was prepared to achieve sustained drug release from a single application. Biofilm eradication of flucloxacillin and lactoferrin under this local delivery system was evaluated by CFU counting.
Results: The findings showed that the combination of flucloxacillin and lactoferrin significantly enhanced biofilm eradication efficacy. Co-administration of 80 mg/mL lactoferrin with flucloxacillin at concentrations of 20 µg/mL and 200 µg/mL resulted in a >6-log reduction in media CFU within two days, and mature biofilms on the metal coupon surfaces were almost completely eradicated after 3 days. This enhanced biofilm killing was observed in subsequent experiments with the Hydrogel-Niosome delivery system ($P < 0.05$), providing an effective approach for delivering lactoferrin and flucloxacillin to biofilms *in vivo*.
Conclusion: Lactoferrin can significantly enhance biofilm eradication in synergy with flucloxacillin, highlighting its potential in treating staphylococcal osteomyelitis.

P27. From Identification to Pathogenesis: Unveiling the Biofilm-Forming Potential of Intratumoral Fungi and Its Therapeutic Implications

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The intratumoral mycobiome is an emerging contributor to cancer biology. To identify key fungal players, we performed an integrated analysis comparing tumor (n=130) with paired normal tissues (n=60). Fungal sequences were profiled from metagenomic data and validated via host RNA-seq deconvolution, revealing a significant enrichment of specific fungi in tumors. Critically, the presence of fungal hyphae within tumor niches was directly confirmed by histopathological staining (IHC and GMS). These findings robustly establish a tumor-resident fungal community.
Building on this discovery, we hypothesize that these intratumoral fungi form biofilms, a protected multicellular state that could be a fundamental mechanism driving their persistence and therapeutic resistance in the harsh tumor microenvironment. Our proposed research aims to directly test this hypothesis. We will isolate these identified fungi and characterize their biofilm-forming capacity under conditions mimicking the tumor niche (e.g., hypoxia). Subsequently, we will investigate the biofilm-associated antimicrobial and chemoresistance profiles, and dissect the underlying molecular mechanisms through transcriptomic analysis. This study bridges the gap between mycobiome identification and functional pathogenesis, offering novel insights into how intratumoral fungi may shield themselves and contribute to cancer therapy failure. Targeting these biofilms could represent a ground-breaking adjuvant strategy.

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