

# 2026 EUROBIOFILMS INTERNATIONAL CONGRESS

GUIMARÃES  
PALÁCIO VILA FLOR  
17<sup>TH</sup>–20<sup>TH</sup> JUNE 2026



Universidade do Minho  
Escola de Engenharia

**U. PORTO**  
FEUP FACULDADE DE ENGENHARIA  
UNIVERSIDADE DO PORTO





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Welcome to EuroBiofilms 2026!

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Guimarães, Portugal

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# CONFERENCE INFORMATION

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## Conference Participation

Eurobiofilms 2026 brings together 272 participants from 24 countries, reflecting the international and multidisciplinary nature of the biofilm research community. Participants include researchers, clinicians, students, industry representatives, and professionals working across fundamental, translational, technological, and clinical aspects of biofilm science.

## Scientific Programme

The scientific programme has been designed to cover the diversity of the biofilm field, including fundamental microbiology, biofilm characterization, modulation and prevention strategies, translational research, clinical applications, and emerging technologies. The programme includes keynote lectures, invited sessions, oral communications, flash presentations and poster sessions. Participants are encouraged to actively engage in the sessions, ask questions, and use the conference as an opportunity to exchange ideas and establish collaborations. Sessions will take place according to the schedule provided in the scientific programme. Parallel sessions will be held in the indicated auditorium, and participants are advised to check the programme regularly for session locations and timing.

## Poster Sessions

There will be two poster sessions during the conference. Posters will be displayed on the balcony of 2<sup>nd</sup> floor. Poster presenters must place their posters in the session assigned to them, following the poster number and poster session indicated in the programme and in the exhibition place. Presenters should ensure that their posters are mounted from 9 am to 12 am and removed at the end of the assigned display period (from 5 pm to 7 pm). Unclaimed posters left hanging after the removal deadline will be discarded. Mounting supplies will be provided. All participants are encouraged to visit the poster area and interact with poster presenters, particularly early-career researchers and students.



# CONFERENCE INFORMATION

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## Awards

Eurobiofilms 2026 will include seven awards recognizing scientific excellence in oral and poster presentations. Awards will be attributed to the best two oral presentations and to the best five posters.

## Certificates

Attendance certificates will be provided digitally and sent by email after the conference.

## Wi-Fi

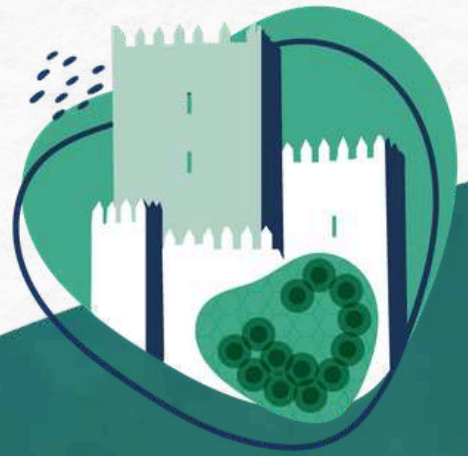
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## Social Programme

The social programme has been organized to provide opportunities for informal networking and interaction among participants.

A **welcome reception cocktail** will take place after the afternoon sessions on the first day of the conference. The **conference dinner** will be held at Quinta da Granja (41.511015, -8.382286). Transportation will be provided from Palácio Vila Flor at 18:30, with return transportation scheduled for 23:00. Participants will also have the opportunity to join a **guided walking tour** through the historic city of Guimarães, led by conference staff. Conference participants may also use the city cable car to visit Penha hill overlooking Guimarães with panoramic views of the city and the surrounding landscape. A 50% discount is available for conference participants.



# T

Welcome

# MESSAGE



# WELCOME MESSAGE

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On behalf of the **Organizing Committee**, it is our pleasure to welcome you to EuroBiofilms 2026, which will take place in the historic city of **Guimarães**, Portugal, from June **17th to 20th, 2026**.

We are delighted to bring together our multidisciplinary community of **researchers, academics, and clinicians** from Europe and around the world to share the latest discoveries, exchange innovative ideas, and strengthen our collective efforts to better understand and combat **biofilm-related infections**.

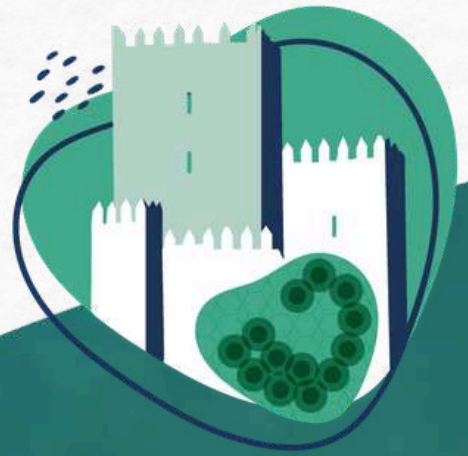
The **scientific program** has been carefully designed to reflect the diversity and dynamism of the field, from **fundamental microbiology to translational and clinical research**, highlighting the latest advances in **biofilm characterization, modulation and prevention**. Your active participation and unique perspectives will be essential as we address the complex evolving challenges posed by biofilms.

**Guimarães**, a **UNESCO World Heritage** site and the **birthplace of the Portuguese nation**, offers a beautiful and inspiring environment for our discussions. We encourage you to take this opportunity to explore the city's rich history while engaging with colleagues and creating lasting collaborations and new ideas for the future. We look forward to welcoming you to Guimarães!

The Organizing Committee,

**Nuno Azevedo**

**Joana Azeredo**



# 2

VENUE



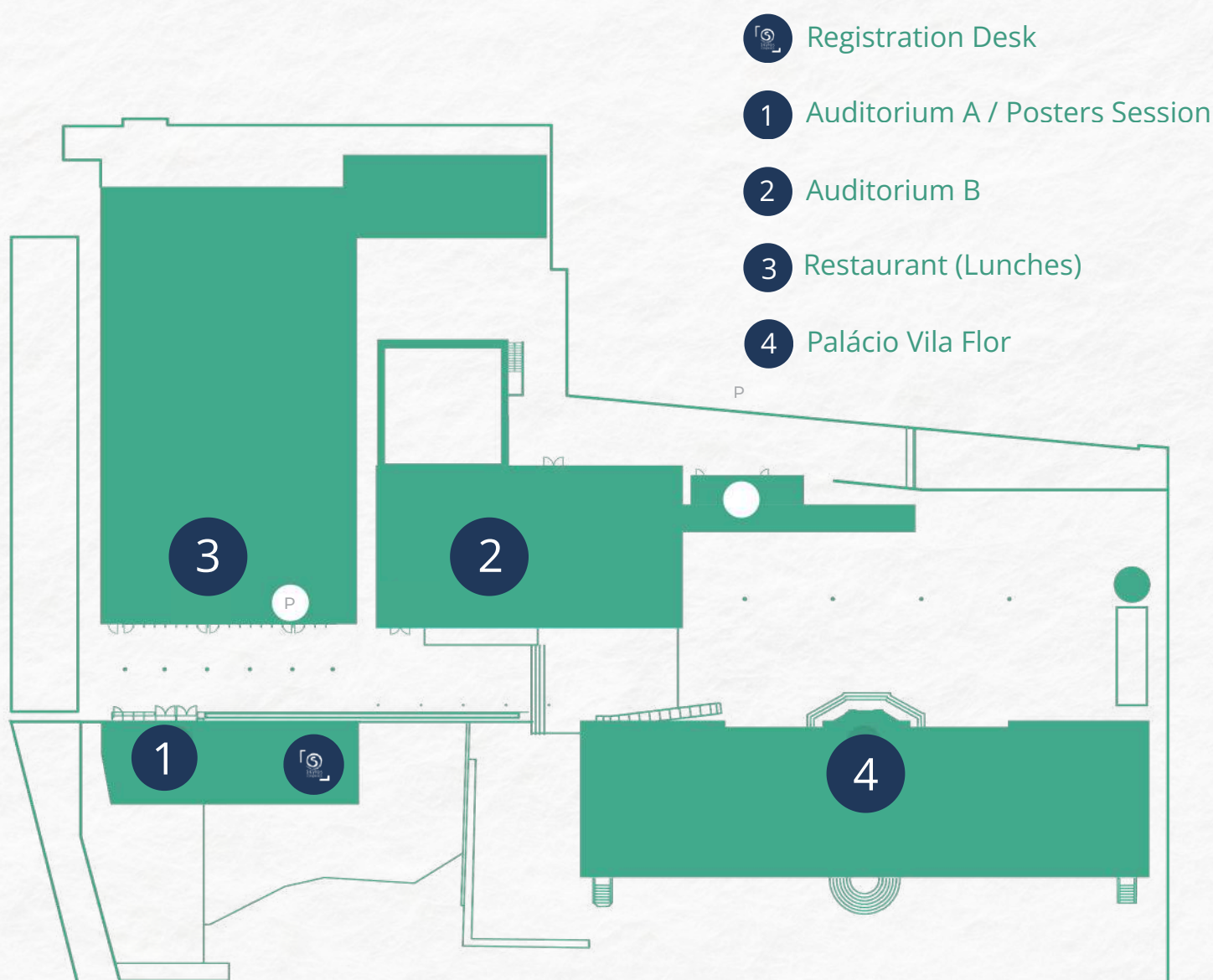
# VENUE

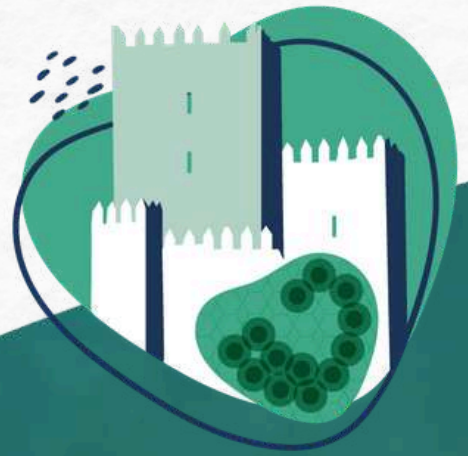
## Venue and Dates

Eurobiofilms 2026, 9th European Conference on Biofilms, will take place from 17–20 June 2026 at Palácio Vila Flor, in Guimarães, Portugal.

Palácio Vila Flor is a magnificent 18th century palace located in the heart of Guimarães, Portugal. Today, the palace serves as the centerpiece of Centro Cultural Vila Flor, one of Portugal's premier cultural venues since 2005.

**Address:** Av. D. Afonso Henriques, 701, 4810-431 Guimarães





# 3

# ORGANIZATION



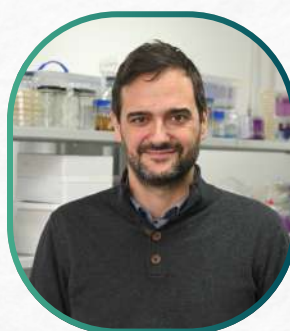
# ORGANIZATION

EuroBiofilms 2026 is an ESCMID Biofilm Study Group conference, locally organized in Portugal by the University of Minho and the University of Porto, with the partnership of TecMinho and the Câmara Municipal de Guimarães.



**JOANA AZEREDO**

University of Minho,  
Centre of Biological  
Engineering



**NUNO AZEVEDO**

University of Porto,  
Faculty of Engineering,  
LEPABE-ALiCE



**ANABELA BORGES**

University of  
Porto, Faculty of  
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University of  
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**LUÍS D. R. MELO**

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HELENA BUJDAKOVA



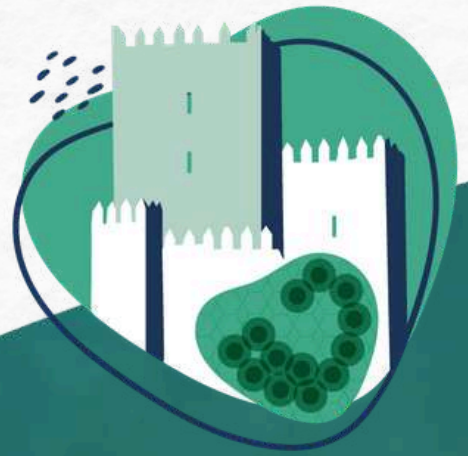
MARGAREET C. VOS



MARIA DOLORES  
MACIA ROMERO



LUIS DR MELO



4+

Invited  
SPEAKERS



# INVITED SPEAKERS

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**ROBIN PATEL**

KEYNOTE SPEAKER

Robin Patel, M.D., is an internationally recognized leader in clinical microbiology and infectious diseases. As a professor and researcher at the Mayo Clinic, she has made pioneering contributions to the study of biofilm-associated and prosthetic joint infections, as well as to the development of innovative diagnostic approaches, including sonication and metagenomic techniques. With more than 450 scientific publications, Dr. Patel has also played a major role in antimicrobial resistance research, mentorship, and international scientific leadership, including serving as President of the American Society for Microbiology (ASM). Her career is distinguished by the integration of research, clinical practice, and innovation to improve patient care.



**JOÃO XAVIER**

KEYNOTE SPEAKER

Systems biologist Joao Xavier combines experimental and computational approaches to study diverse problems relevant to cancer: how the microbiome influences cancer and cancer treatment, how cancer cells metastasize, and how metabolic fluxes command the behavior of living cells.

# INVITED SPEAKERS

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CÉU FIGUEIREDO

Céu Figueiredo, PhD, is a Group Leader at i3S/Ipatimup and Associate Professor at the Faculty of Medicine of the University of Porto. Her research focuses on the pathogenesis of *Helicobacter pylori* infection, particularly the role of bacterial virulence factors, host genetic susceptibility, and gastric microbiota in gastric cancer development. She has authored more than 130 peer-reviewed publications, 10 book chapters, and holds two international patents. Dr. Figueiredo has coordinated and participated in several national and international research projects funded by organizations including FCT, the European Commission, and Horizon Europe.



DIRK BUMANN

Prof Dirk Bumann is Professor of Infection Biology at the Biozentrum of the University of Basel. His research group has worked on mechanisms of bacterial infection in animal models and human patients. In recent years, the lab has focused on single cell activities of pathogens in host tissues as a basis for developing novel anti-infective strategies. The main experimental approaches include flow cytometry, mass spectrometry and two-photon tomography.



HOLGER ROHDE

Holger Rohde is a Senior Physician, Head of Research Group, and specialist in Microbiology, Virology, and Infectious Disease Epidemiology. His work focuses on clinical microbiology, infectious diseases, and translational research, contributing to advances in the understanding and management of microbial infections.



# INVITED SPEAKERS

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JEAN-MARC GHIGO

Professor at the Institut Pasteur and Head of the Genetics of Biofilms Unit, his research focuses on the molecular and genetic mechanisms of bacterial processes and biofilm formation. After completing his PhD at the Institut Pasteur and postdoctoral training at Harvard Medical School, he established a pioneering research group on bacterial biofilms at the Institut Pasteur in 2002.



JING YAN

Jing Yan is an Assistant Professor of Molecular, Cellular and Developmental Biology at Yale University and a member of the Quantitative Biology Institute (Qbio). He earned his B.S. from Peking University and his Ph.D. in Materials Science and Engineering at the University of Illinois at Urbana-Champaign, where he worked on active colloidal materials. He later transitioned to microbiology and quantitative biology during a postdoctoral fellowship at Princeton University, studying bacterial biofilms. Since 2019, his research has focused on quantitative and interdisciplinary approaches to microbiology at Yale.



LINDSAY HALL

Professor Lindsay Hall is Chair of Microbiome Research and a Wellcome Investigator. Her research focuses on understanding how host-associated microbiomes, particularly in early life, influence health, development, and disease risk, including chronic conditions such as cancer. She leads an international, multidisciplinary team studying microbial colonisation in pregnancy and infancy and its long-term physiological impact. Her work is widely funded and complemented by a strong commitment to public engagement and science communication.



# INVITED SPEAKERS

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HENRIETTE LYNG  
RØDER

Henriette Lyng Røder is an Associate Professor working in Food Microbiology, Gut Health, and Fermentation. Her research focuses on microbial processes in food systems and their relevance to gut health and human well-being, with particular interest in fermentation and the gut microbiome.



TOM COENYE

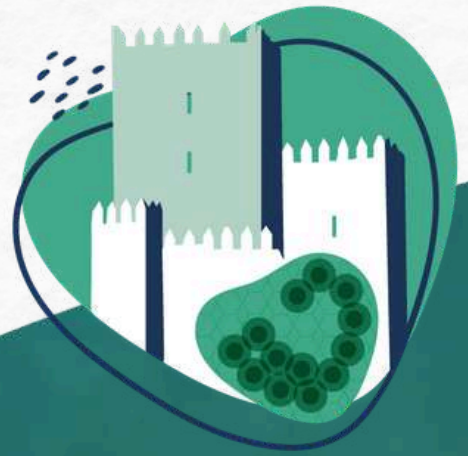
Tom Coenye obtained his MSc in Biochemistry and PhD from Ghent University, where he focused on the taxonomy and diagnosis of *Burkholderia cepacia* complex infections in cystic fibrosis patients. After postdoctoral research at the University of Michigan, he returned to Ghent University to work on bacterial taxonomy and genome-based approaches. He is currently a research professor at the Laboratory of Pharmaceutical Microbiology, where his work focuses on microbial behaviour, biofilms, and chronic infections.



TRISTAN FERRY

Tristan Ferry is an infectious diseases specialist trained at Claude Bernard Lyon 1 University and Hospices Civils de Lyon, where he also completed a PhD and habilitation. He further trained in Geneva, focusing on HIV and bone and joint infections.

He is currently an infectiologist at Hospices Civils de Lyon and heads the regional reference center for complex bone and joint infections, coordinating a multidisciplinary network. His research focuses on innovative treatments for these infections, particularly bacteriophage therapy and local antibiotic strategies. He is actively involved in national and international scientific leadership roles and multicentric clinical trials in infectious diseases.



# 5

# WORKSHOP



# WORKSHOP

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## **In vivo tissue related biofilm infections - necessary translational step for improved clinical biofilm understanding and treatment**

### **The subjects will be:**

- Introduction to animal research
- Chronic wound models
- Lung and Cardiovascular models

The workshop will present a general introduction to animal (biofilm) research, legislation, ethics, statistics and endpoints. Present state-of-the art biofilm models of non-healing wounds, as well as lung and cardiovascular models, contribution to biofilm-host understanding, translational potential and promising treatment candidates. The presentations will be educational and interactive with time for questions and discussions.

### **Coordinators:**



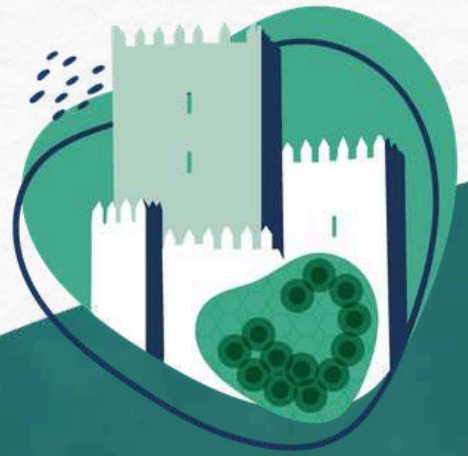
CLAUS MOSER

Department of Clinical  
Microbiology, Copenhagen  
University Hospital



KENDRA RUMBAUGH

Department of Surgery,  
School of Medicine,  
TTUHSC



6

PROGRAM

# PROGRAM



**17 JUNE**  
Wednesday

|                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>09:00 - 10:30</b> | <b>Workshop</b> <span>Auditorium A</span><br>In vivo tissue related biofilm infections - necessary translational step for improved clinical biofilm understanding and treatment                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>10:30 - 11:00</b> | <b>Coffee Break</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>11:00 - 12:30</b> | <b>Workshop</b> <span>Auditorium A</span><br>In vivo tissue related biofilm infections - necessary translational step for improved clinical biofilm understanding and treatment                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>12:30 - 14:00</b> | <b>Lunch</b><br>Reserved exclusively for workshop participants                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>14:00 - 14:30</b> | <b>Opening Session</b> <span>Auditorium A</span>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>14:30 - 15:30</b> | <b>Keynote 1: Microbiome ecology for cancer care</b> <span>Auditorium A</span><br>Invited Speaker: João Xavier                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>15:30 - 17:00</b> | <b>Session 1</b> <span>Auditorium A</span><br><b>Diagnosis of clinical biofilms</b><br><b>Moderators:</b> Helena Bujdakova and Claus Moser<br><b>Invited Speaker:</b> Holger Rohde<br>Precision diagnostics in prosthetic joint infections: emerging technologies and strategies<br>Selected Oral Presentations:<br><b>OP1.1 - Identification of genes involved in aggregate associated antimicrobial tolerance of Pseudomonas aeruginosa using Tn-Seq guided discovery</b> - Lise Goltermann<br><b>OP1.3 - Antimicrobial resistance profiling and biofilm detection in cystic fibrosis sputum using MX-Raman</b> Callum Highmore<br><b>OP1.4 - mRNA PNA-FISH as a Spatial Transcriptomics Platform for Single-Cell Gene Expression Analysis in Biofilms</b> - Laura Cerqueira |
| <b>17:00 - 19:00</b> | <b>Reception</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

# PROGRAM



**18 JUNE**  
Thursday

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|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>09:00 - 10:30</b> | <div><b>Session 2</b></div> <div>Interactions at the surface</div> <div><b>Moderators:</b> Filipe Mergulhão and Rikke Meyer<br/><b>Invited Speaker:</b> Jean-Marc Ghigo</div> <div>Exploring biofilm formation in the gut symbiont bacteroides thetaiotaomicron</div> <div>Selected Oral Presentations</div> <div><b>OP2.1 - Antiparallel stacking of Csu pili drives Acinetobacter baumannii 3D biofilm assembly -</b><br/>Anton Zavialov</div> <div><b>OP2.2 - Function and spatio-temporal patterning of the Small basic protein (Sbp) in Staphylococcus epidermidis biofilm formation -</b> Michelle Savickis</div> <div><b>OP2.3 - Exploring Type IV Pili Function within Biofilms Using Genetic Code Expansion in Pseudomonas aeruginosa and Acinetobacter baumannii -</b> Clara Dessenne</div> <div><b>OP2.4 - Surface-proximity-dependent reprogramming of biofilm architecture independent of antimicrobial activity -</b> Ana I. Romero</div> |
| <b>10:30 - 11:00</b> | <div>Coffee Break</div> <div>Balcony of 2nd floor</div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>11:00 - 12:30</b> | <div><b>Session 3</b></div> <div>Innovative biofilm methods: pushing the state of the art</div> <div><b>Moderators:</b> Luís D. R. Melo and Kendra Rumbaugh<br/><b>Invited Speaker:</b> Tom Coenye</div> <div>Innovating the biofilm toolbox: synthetic in vivo-like media meet advanced analytics</div> <div>Selected Oral Presentations</div> <div><b>OP3.1 - A Physiologically Relevant In Vitro Model for Staphylococcus aureus Biofilms in Prosthetic Joint Infection -</b> Yasser Dghoughi</div> <div><b>OP3.2 - Novel techniques for studying Legionella pneumophila attachment -</b> Craig Billington</div> <div><b>OP3.3 - Using the Galleria mellonella model to study burn wound biofilm formation -</b> Ronan R McCarthy</div> <div><b>OP3.4 - Non-interventional real-time monitoring of biofilm formation provides new insights in extracellular matrix growth and maturation -</b> Agneta Richter-Dahlfors</div>                         |
| <b>12:30 - 14:00</b> | <div>Lunch</div> <div>Restaurant</div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

# PROGRAM



**18 JUNE**  
Thursday

## Poster Session 1

Balcony of 2nd floor

### Session 1 : Diagnosis of clinical biofilms

**P1.1 - Biofilm-Forming Pathogens in Orthopaedic Implant-Associated Infections: A Clinical Study from Indonesia** - Titik Nuryastuti

**P1.2 - XpertBiofilm: A simple diagnostic platform reveals shear stress as critical in biofilm development.**  
Núria Blanco-Cabra

**P1.3 - Developing a Multispecies Approach for Assessing Biofilm Formation and Control in Aqueous-Based Cooling Fluids** - Michaela E Morris

**P1.4 - Joint Infection Multiplex PCR Panel Repurposing for Sonication Fluid Analysis in Orthopedic Implant-Associated Infections** - Emilia-Ruxandra BĂLULESCU

**P1.5 - Infrared Spectroscopy for Rapid Diagnosis of Central Line Infection** - Amy R. Crisp

**P1.6 - Streptococcus agalactiae, a frequent but not so well-known cause of bone and joint infections**  
Simon Jamard

**P1.7 - Low toxicity of flavonoid-based antifouling candidates on marine bacteria** - Ana Raquel Rodrigues

**P1.8 - Identifying clinically relevant Candida isolates for evaluation of dendritic antifungals**  
Eloísa García-Porcel

**P1.9 - Diagnostic Yield of Culture of Synovial Fluid, Periprosthetic Tissue and Sonicate Fluid in Patients Undergoing Debridement, Antibiotics and Implant Retention for Periprosthetic Joint Infection**  
Judith Alvarez Otero

**P1.10 - Mining Novel Antimicrobial Peptides from Hydrocarbon-Contaminated Environments: Targeting ESKAPE Pathogens and Biofilms** - Jordanne Maybin

**P1.11 - Preliminary study on the application of hyperspectral imaging in the assessment of biofilm formed by Coagulase-Positive Staphylococci (S. aureus) and Coagulase-Negative Staphylococci (CoNS) collected from cardiac implantable electronic device-related infections** - Beata Sarecka-Hujar

14:00 - 14:30

# PROGRAM



**18 JUNE**  
Thursday

## Poster Session 2

Balcony of 2nd floor

### Session 2 : Interactions at the surface

**P2.1 - Is 2D and 3D-OCT imaging information comparable for Pseudomonas fluorescens biofilms grown in a CDC reactor?** - Luís Costa

**P2.2 - Mechanosensitive regulation of biofilm dynamics by QseBC in Klebsiella pneumoniae** - Caroline MASSIT

**P2.3 - Contact-killing efficacy of chitosan-based hydrogels against staphylococci for wound dressing application** - Adèle Huc

**P2.4 - Quantifying the Role of Nutrient Adsorption in Early Biofilm Expansion** - Jacobus Brink

**P2.5 - Linking biofilm dispersion to membrane vesicle production in Pseudomonas aeruginosa** Mizuki Kanno

**P2.6 - Sedimentation Contributed to Higher Biofilm Thickness and Biofilm Structure Heterogeneity** Francisca Ferreira

**P2.7 - Plant-Inspired Microtopographies to Reduce E. coli Binding on Food Contact Surfaces** Fábio Carvalho

**P2.8 - Selective Capture of Microplastics by Environmental Biofilm-Forming Bacteria Isolated from Plastic Debris** - Manuel Romero

**P1.9 - Diagnostic Yield of Culture of Synovial Fluid, Periprosthetic Tissue and Sonicate Fluid in Patients Undergoing Debridement, Antibiotics and Implant Retention for Periprosthetic Joint Infection** Judith Alvarez Otero

**P2.9 - From Antioxidant to Antibiofilm Agent: Ascorbic Acid Outperforms Citric Acid Against Early and Mature Biofilms** - Inês Mó

**P2.10 - Shear-Driven Biofilm Detachment: Time-Resolved Analysis of Size Spectra in Staphylococcus epidermidis Biofilm** - Dibyangshee Singh

**P2.11 - Domiphen Bromide-Functionalized 3D-Printed PCL Surfaces Prevent Biofilm Formation by Clinical Biliary Isolates** - Claudia Aiello

**P2.12 - Interplay between coaggregation and extracellular metabolites shapes biofilm formation** Ana Cristina Afonso

**P2.13 - Glucose-gentamicin interplay governs surface-associated motility and biofilm dynamics in Pseudomonas aeruginosa isolate HRW.1-S3** - Saurav Kumar Saha

**P2.14 - Bioglass-based Membranes Incorporating an Ionic Liquid for Cartilage Tissue Regeneration** I. Almeida

14:00 - 14:30

# PROGRAM



**18 JUNE**  
Thursday

## Poster Session 3

Balcony of 2nd floor

### Session 2 : Innovative biofilm methods: pushing the stage of art

**P3.1 - Monitoring intracellular antibiotic concentrations in real-time using allosteric biosensors**  
Daria Fleckenstein

**P3.2 - A High-Throughput, Droplet-Based Culture Method for Reliable Assessment of Biofilm Growth on Various Substrates** - Mihyun Lee

**P3.3 - Evaluation of a depolymerase-encoding bacteriophage against Shiga toxin-producing Escherichia coli biofilms under dynamic flow** - Nicole Canales-Huerta

**P3.4 - Pseudomonas aeruginosa Biofilm Dynamics Under Ocular Oscillating Flow Conditions**  
Niamh Corkery-Hayward

**P3.5 - Elimination of bacterial biofilm models using bacteriophages and their lytic enzymes**  
Moša Marek

**P3.6 - Phage-based therapeutics against bacterial biofilms in preclinical studies** - Fuglúk Vítézslav

**P3.7 - Decoding the EPS matrix: A multidisciplinary approach for understanding the recalcitrance of industrial biofilms** - Sofia R. Cruz

**P3.8 - Why the biofilm field must move beyond viable plate counts: limitations and path forward for regulatory decision-making** - Céilia Fortuna Rodrigues

**P3.9 - The NADH:NAD<sup>+</sup> Ratio Influences E. coli K-12 MG1655 Surface Attachment and Biofilm Growth**  
Qonita Afinanisa

**P3.10 - Mapping key transcriptional signatures of Listeria monocytogenes persistence in food industry biofilms using a mRNA PNA-FISH strategy** - Rui Macedo

**P2.10 - Shear-Driven Biofilm Detachment: Time-Resolved Analysis of Size Spectra in Staphylococcus epidermidis Biofilm** - Dibyangshee Singh

**P2.11 - Domiphen Bromide-Functionalized 3D-Printed PCL Surfaces Prevent Biofilm Formation by Clinical Biliary Isolates** - Claudia Aiello

**P3.11 - Enzymatic breakdown of biofilm matrix to allow flow cytometry viability analysis of Clostridium beijerinckii cells** - Hélène Velly

**P3.12 - Evolution of biofilm cells viability in fixed bed biofilm reactor during isopropanol butanol ethanol fermentation with Clostridium beijerinckii DSM 6423** - Hélène Velly

**P3.13 - Multiplexed antimicrobial susceptibility testing in microfluidic drops** - Connie B. Chang

**P3.14 - A bioluminescence quantitative assay evaluation to determine the biomass of attached bacteria in surface biofilm formation**  
Francesca Tolio

14:00 - 14:30

# PROGRAM



**18 JUNE**  
Thursday

**14:00 - 14:30**

**P3.15 - Biofilm Dynamics in Cystic Fibrosis: Application of NAM-FISH in Air3Gel, a 3D airway mucus model**  
Isadora Maia

**P3.16 - mRNA PNA-FISH Profiles Spatial Gene Expression Dynamics in Legionella pneumophila Biofilms**  
Ana Barbosa

**P3.17 - Antibiotic-resistant and tyramine-producing Enterococcus faecalis isolated from cheese form robust biofilms on stainless steel** - Natalia Merino Almalé

**P3.18 - Fluorescent Imaging Sheds Light on Mycobacterial Biofilm Architecture In Vitro and Ex Vivo**  
Nelli Vahvelainen

**P3.19 - Multiscale Investigation of Biofilm Dynamics and Remediation in Porous Media** - H      Velly

**P3.20 - A transcriptomic dispersal fingerprint of Pseudomonas aeruginosa** - Xavier Bertran i Forga

**P3.21 - Development of a screening strategy for biofilm-forming bacteria isolated from cheese processing environments** - Livia Balaguer

## Poster Session 5

### Session5 : Oral Biofilms

**P5.1 - Modulating Oral Biofilms Through Prebiotics** - Jasmin Clark

**P5.2 - Evaluating LACTAM as a novel anti-biofilm strategy for oral healthcare** - William Johnston

**P5.3 - Multiscale mechanical properties of S. mutans biofilms** - Mrunmayee Joshi

**P5.4 - Biophysical Characterisation of the Physical Mode of Action of Sodium Bicarbonate** - Rob Howlin

**P5.6 - Partitioned Lipid A Modification Pathways Link Outer Membrane Vesicle Biogenesis to Biofilm Development and Virulence in Porphyromonas gingivalis** - Beichang Zhang

**P5.7 - Modelling Microbial Biofilms of Oral Health States for Enhanced Pre-clinical Research** - Katie Grey

**P5.8 - Antimicrobial and Antibiofilm Efficacy of Chitosan-Loaded Monarda Essential Oil Against Mono- and Mixed-Species Oral Biofilms of Staphylococcus aureus and Candida albicans** - Divya Varshney

**P5.9 - Ex-vivo analysis of saliva-derived biofilms reveals antifungal influence on bacterial community composition in the presence and absence of Candida albicans** - Mark Butcher



**18 JUNE**  
Thursday

14:00 - 14:30

**Session 10 : Biofilm tolerance and resistance**

**P10.1 - Enhancing bacteriophage activity against *Pseudomonas aeruginosa* biofilms formed by clinical isolates through directed evolution** - Sopiko Sturua

**P10.2 - Novel Lytic Bacteriophages for Combatting Biofilm-Associated Infections by *Pseudomonas aeruginosa* and *Staphylococcus* spp** - Natia Karumidze

**P10.3 - Transient Antibiotic Efficacy Against *Enterococcus faecalis* Biofilms in an In Vitro Vascular Graft Infection Model** - Rebekka Faber Karpatschof

**P10.4 - 5-Fluoro-cytosine: an antimetabolite with antibiofilm property against uropathogenic *Escherichia coli* strains** - Giulia Ghezzi

**P10.5 - Glycopeptide and lipoglycopeptide-mitomycin C conjugates for targeted delivery to biofilm infections**

Alif Firman Firdausy

**P10.6 - A Promising Flavonoid Scaffold for Anti-Biofilm Agent Development** - Mihaela Savu

**P10.7 - The role of antimicrobial efflux in shaping biofilm spatial structure** - Valerie Jaut

**P10.8 - When Cleaning Isn't Enough: Superior Anti-Biofilm Activity of Disinfectants over Detergents against MDR Clinical Isolates** - Beata Leeb-Zatorska

**P10.9 - Modulation of the agr Quorum Sensing System by Light-Activated Nanocomposite with Erythrosine B in *Staphylococcus aureus*** - Larysa Bugyna

**P10.10 - Regulatory Roles of Novel Small Non-Coding RNAs in Biofilm Formation, and Antimicrobial Resistance in Strong Biofilm-Forming *Acinetobacter Baumannii* Clinical Isolate** - Tilaye Shibbiru Mengistu

**P10.11 - Evaluation of the antifungal activity of dendritic systems against *Candida tropicalis* biofilms**

Irene Heredero-Bermejo

**P10.12 - In vitro activity of dendritic systems against MRSA and MSSA biofilms**

Natalia Gómez-Casanova

**P10.13 - Energy, Efflux and Resistance in Bacteria** - Iremide Sanyaolu

**P10.14 - *Candida glabrata* biofilms under vaginal conditions: impact of pH and correlation with antifungal resistance** - Bruna Gonçalves

**P10.15 - Cold plasma opens antibiotic susceptibility windows in implant-associated *Staphylococcus aureus* biofilms** - Thomas P. Thompson

**P10.16 - Hybridisation of ciprofloxacin with geraniol disrupts *Staphylococcus aureus* biofilms and overcomes fluoroquinolone resistance** - Miguel M. Leitão



**18 JUNE**  
Thursday

**14:00 - 14:30**

**P10.17 - Deciphering the Mechanisms of Synergistic Repeated Photoactivation of a Naturally Derived Combination Against Dual-Species *Pseudomonas aeruginosa*-*Staphylococcus aureus* Biofilms**  
Ariana S.C. Gonçalves

**P10.18 - Network-level divergence in cyclic di-GMP signalling drives ecological versatility in *Acinetobacter baumannii*** - Lyuboslava G Harkova

**P10.19 - Focus on endoscope reprocessing: Peracetic acid efficacy against biofilms of clinically relevant ESKAPE pathogens** - Anja M. Richter

**P10.20 - Analysis of molecular regulation and quorum sensing in biofilms of clinical isolates of *Klebsiella pneumoniae* and contribution of photodynamic inactivation in communication of bacteria within biofilms**  
Jaroslava Dekkerová

**P10.21 - Evaluation of antibiotic resistance in autoaggregated *Staphylococcus aureus* using a magnetic levitation-based experimental system** - Dovlet Rejepov

**P10.22 - The global transcriptome response of *Pseudomonas aeruginosa* to epoxytiglic acid EBC-1013**  
Jacob O. Evans

**P10.23 - Natural mineral waters as adjunctive strategies against multidrug-resistant pathogens in biofilm-associated diabetic foot ulcers** - Ana Rita Pinto

**P10.24 - Experimental evolution of antibiotic resistance in the ex vivo pig lung biofilm model**  
Charlotte Cornbill

**P10.25 - Persister cell emergence occurs beyond mature stages of *Cryptococcus* biofilms**  
Gabriel Rezende Pimenta

**P10.26 - Phytochemicals as alternative strategies to traditional biocides for controlling bacterial biofilms in hospital disinfection** - Mariana Sousa

**P10.27 - Cefiderocol Efficacy in *Pseudomonas aeruginosa* Biofilms: Impact of Iron Limitation, Siderophore Transport and Metallo- $\beta$ -lactamase Production** - María Fernández-Billón

**P10.28 - Spatiotemporal development of antibiotic persistence in *Vibrio cholerae* biofilms depends on emergent microenvironments** - Dibya Saha

**P10.29 - Disrupting Biofilm-Associated Genes Reduces Mycobacterial Virulence** - Emilia Lintilä

**P10.30 - Characterization of a novel putative amyloid identified in mycobacterial biofilm** - Juliaana Katto

**P10.31 - Disruption of *Candida* spp. Biofilms by Insect-Derived Peptides: An In Vitro Study**  
Catarina A. M. Sousa

# PROGRAM



**18 JUNE**  
Thursday

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| <p><b>14:30 - 16:00</b></p> | <p style="text-align: right;"><b>Auditorium A</b></p> <p><b>Parallel Session 4</b><br/><b>Biophysical, mathematical and computational methods applied to biofilms</b></p> <p><b>Moderators:</b> Anabela Borges and Henriette Lyng Røder<br/><b>Invited Speaker:</b> Jing Yan<br/>Single-cell imaging of microbial communities across different spatial and temporal scales</p> <p>Selected oral presentations</p> <p><b>OP4.1 - Molecular rules of engagement: how self-associating autotransporters shape biofilm architecture</b><br/>Begona Heras</p> <p><b>OP4.2 - DNA from genome to critical extracellular viscoelastic biofilm matrix component through higher order structural changes</b> - Thomas Seviour</p> <p><b>OP4.3 - Polysaccharide components provide the major diffusion barrier for nano-sized objects in glycan-based biofilms</b> - Stefanie Barbirz</p> <p><b>OP4.4 - Spatiotemporal Staphylococcus aureus Biofilm Dynamics in a 3D in vitro Platform</b><br/>Silvia Cometta</p> |
| <p><b>14:30 - 16:00</b></p> | <p style="text-align: right;"><b>Auditorium B</b></p> <p><b>Parallel Session 5</b><br/><b>Oral biofilms</b></p> <p><b>Session Organizers:</b> Bastiaan Krom, Nick Jakubovics and Hyun (Michel) Koo</p> <p>Selected oral presentations</p> <p><b>OP5.1 - Intro/oral biofilm initiative</b> - Bastiaan Krom</p> <p><b>OP5.2 - Laser-assisted bioprinting to characterize complex biofilms</b> - Szymon Szafranski</p> <p><b>OP5.3 - Hormones as host-derived modulators of biofilms</b> - Aurea Simon-Soro</p> <p><b>OP5.4 - Hacking oral biofilms with smart nanotechnologies and microrobots</b> - Hyun (Michel) Koo</p>                                                                                                                                                                                                                                                                                                                                                                                |
| <p><b>16:00 - 16:30</b></p> | <p><b>Coffee Break</b></p> <p style="text-align: right;"><b>Balcony of 2nd floor</b></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

# PROGRAM



**18 JUNE**  
Thursday

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| <p><b>16:30 - 18:00</b></p> | <p style="text-align: right;"><b>Auditorium A</b></p> <p><b>Parallel Session 6</b><br/><b>Multispecies biofilms</b></p> <p><b>Moderators:</b> Lindsay Hall and Gordon Ramage<br/><b>Invited Speaker:</b> Henriette Lyng Røder<br/>Behaviour and adaptation in multispecies biofilms are defined by neighbours</p> <p>Selected oral presentations</p> <p><b>OP6.1 - Metabolic Evasdropping: Amoeba-derived Acetaldehyde drives Bacterial Flight from Amoeba Predation</b> - Ilana Kolodkin-Gal</p> <p><b>OP6.2 - Development and characterization of a mixed Pseudomonas aeruginosa-Mycobacterium abscessus biofilm model for the evaluation of clinically relevant antibiotics and new antibiofilm agents for the treatment of CF-related co-infections</b> - Chloé Garcia Labaig</p> <p><b>OP6.3 - Intracellular survival of Pseudomonas aeruginosa and Staphylococcus aureus in a physiologically relevant cystic fibrosis in vitro model: implications for antibiotic invasion and infection relapse</b><br/>Filipa Ribeiro</p> <p><b>OP6.4 - Reduced efficacy of efflux pump inhibitors as adjuvant therapy in polymicrobial biofilms</b><br/>Brogan Richards</p>                                                                                                                                   |
| <p><b>16:30 - 18:00</b></p> | <p style="text-align: right;"><b>Auditorium B</b></p> <p><b>Parallel Session 7</b><br/><b>Session 7 – Cross-topic</b></p> <p><b>Moderators:</b> Maria do Céu Figueiredo and Jean-Marc Ghigo</p> <p>Selected oral presentations</p> <p><b>OP7.1 - Microplastic particles and plastic additives promote biofilm formation, virulence and antibiotic resistance in Vibrio cholerae</b> - Kerstin Thriene</p> <p><b>OP7.2 - Pathogenic bacteria are electrogenic and use catalytically active DNA structures for extra-cellular electron transfer and protection against oxidative stress</b> - Rikke Louise Meyer</p> <p><b>OP7.3 - Efflux-mediated cross-resistance and collateral sensitivity between antibiotics and biocides during surface-associated growth of Pseudomonas aeruginosa</b> - Julien Thebing</p> <p><b>OP7.4 - Global transcriptomic profiling of Pseudomonas aeruginosa PAO1 dynamic biofilm</b><br/>Théo OSMOND</p> <p><b>OP7.5 - A multimodal therapy integrating ultrasound-stimulated microbubbles, antibiofilm agents, and antibiotics against early-stage and mature biofilms</b> - Aaron Crowther</p> <p><b>OP7.6 - Sprayable hyaluronic acid-polyarginine supramolecular films for rapid, antibiotic-free prevention of wound and device biofilms</b> - Skander Hathroubi</p> |
| <p><b>18:30</b></p>         | <p><b>Conference Dinner</b></p> <p style="text-align: right;"><b>Quinta da Granja</b></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

# PROGRAM



**19 JUNE**  
Friday

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| <p><b>09:00 - 10:30</b></p> | <p style="text-align: right;"><b>Auditorium A</b></p> <p><b>Session 8</b><br/><b>Beneficial biofilms (microbiomes)</b></p> <p><b>Moderators:</b> Ângela França and Phil Stewart<br/><b>Invited Speaker:</b> Lindsay Hall<br/>Beneficial biofilms - a focus on the early life gut microbiota</p> <p>Selected oral presentations</p> <p><b>OP8.2 - Evaluation of gene expression markers for diagnosis of bacterial vaginosis: a Gardnerella-specific transcriptional profile</b> - Lucia Sousa</p> <p><b>OP8.3 - Polymicrobial communities drive biofilm formation and exacerbate host responses in a 3D human bladder microtissue model</b> - Ramon Garcia Maset</p> <p><b>OP8.4 - Beyond Single Pathogens: What Mixed Co-Infections Reveal About Chronic Lung Disease</b><br/>Eduard Torrents</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <p><b>10:30 - 11:00</b></p> | <p><b>Coffee Break</b> <span style="float: right;"><b>Balcony of 2nd floor</b></span></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <p><b>11:00 - 12:30</b></p> | <p style="text-align: right;"><b>Auditorium A</b></p> <p><b>Session 9</b><br/><b>Flash Presentations</b></p> <p><b>Moderators:</b> Luciana Gomes and Robin Patel</p> <p>View Flash Presentations</p> <p><b>FP1 - Advancing Biofilm Innovation: Building Consensus in Regulatory Decision Making</b> - Célia Rodrigues</p> <p><b>FP2 - Nitrate respiration regulates Escherichia coli biofilm morphogenesis through a redox-responsive diguanylate cyclase</b> - Alberto J. Martín-Rodríguez</p> <p><b>FP3 - Exposure to emerging chemicals reshapes biofilms and promotes antibiotic tolerance</b><br/>Ana Rita Pereira</p> <p><b>FP4 - Antigen–Antibody-Based Early Warning System for Detecting EPS Biomarkers During Biofilm Initiation</b> - Sandeep Rellegadla</p> <p><b>FP5 - Eradication of Listeria monocytogenes biofilm using the combined treatment with phytochemicals, blue light and sonication</b> - Magdalena Olszewska</p> <p><b>FP6 - Evaluation of amoxicillin and ciprofloxacin activities against Enterococcus faecalis biofilms in catheter-associated urinary tract infections using a dynamic in vitro pharmacokinetic-pharmacodynamic flow model</b> - Desiye T. Tegegne</p> <p><b>FP7 - Synergistic effect of ceftolozane/tazobactam and phages in biofilm-associated respiratory infections caused by carbapenem-resistant Pseudomonas aeruginosa</b> - Laia Fernandez-Barat</p> <p><b>FP8 - Effect of silver on the surface properties of Zn/Ag for use in pin tract infections and the impact of a blood conditioning film on antimicrobial efficacy</b> - Kathryn Whitehead</p> <p><b>FP9 - MicroICS demonstrates the predictive value of biofilm structure</b> - Jerica Sabotič</p> |

# PROGRAM



**19 JUNE**  
Friday

| 12:30 - 14:00 | Lunch                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Restaurant                  |
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|               | <p><b>Poster Session 2</b></p> <p><b>Session 4: Biophysical, mathematical and computational methods applied to biofilms</b></p> <p><b>P4.1 - Systematic studies of bacteriophage confinement in glycan-based biofilms of Erwinia amylovora</b><br/>Stefanie Barbirz</p> <p><b>P4.2 - Understanding the impact of spatial distribution in biofilm communication through agent-based modelling</b> - Joana Vilas Boas</p> <p><b>P4.3 - A modular computational framework for multifunctional antimicrobial peptide prioritization</b><br/>Dinis Duarte Robalo Martins</p> <p><b>P4.4 - Modelling the Mechanical Response of Dry Biofilms under External Loading: A Continuum Approach</b><br/>Luca Grezzi</p> <p><b>Session 6: Multispecies biofilms</b></p> <p><b>P6.1 - Ecological Therapeutic Strategy for Vulvovaginal Candidiasis: Selective Antimicrobial Efficacy of Natural Extract-Based Mucoadhesive Gels in a Simulated Vaginal Microbiota Model</b><br/>Debora Barros Barbosa</p> <p><b>P6.2 - Advanced glycation is a major pathway for extracellular polymeric substances (EPS) production in activated sludge biofilms from biological wastewater treatment</b> - Thomas Seviour</p> <p><b>P6.3 - Lurking in the broiler chickens' environment: Biofilm formation by Salmonella Infantis and multispecies communities</b> - Lisa De Witte</p> <p><b>P6.4 - Kinetic characterization of mono and polymicrobial biofilm formation by Candidozyma auris and associated bacteria</b> - Bruno Bulgarelli Adorno Arantes</p> <p><b>P6.5 - Exploring the Mycobiome of Dutch Dental Unit Waterlines</b> - Michel Hoogenkamp</p> <p><b>P6.6 - Targeting Bacterial Biofilms Using Cordyceps militaris-Mediated Gold Nanoparticles: EPS Disruption and ROS-Driven Mechanisms</b> - Annesha Roy</p> <p><b>P6.7 - Active PLA-Chitosan Coatings to Prevent Food-Related Multispecies Biofilms</b> - Luciana C. Gomes</p> <p><b>P6.8 - Development of a Pseudomonas aeruginosa and Staphylococcus aureus dual-species biofilm model as a screening platform for biofilm dispersion activity</b> - Mathieu Gonzalez</p> <p><b>P6.9 - The Aggregate Advantage: Unravelling Competitive Dynamics in Mixed Species Biofilms</b><br/>Ciarán Dunne</p> <p><b>P6.10 - Bridging the translational gap: Evaluation of a novel dual-drug antimicrobial coating for endotracheal tubes in a physiologically relevant quasi-in vivo airway model</b> - Susana Patrícia Lopes</p> <p><b>P6.11 - Nucleic Acid Mimics - Fluorescence in situ Hybridization (NAMS-FISH) combined with Spectral Imaging (SI) for Detection and Characterization of Gastric Microbiota</b> - Miranda, Sónia</p> | <b>Balcony of 2nd floor</b> |
| 14:00 - 14:30 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                             |



**19 JUNE**  
Friday

**14:00 - 14:30**

**P6.12 - Towards an understanding of the formation of mixed-species biofilms in the food processing industry: Temperature Effects on Biofilm Development in *Listeria monocytogenes*, *Pseudomonas fluorescens*, *Escherichia coli*, and *Salmonella Typhimurium*** - Obaloluwa Praise Akintayo

**P6.13 - Comparative Cross-Scale Imaging of Mono- and Dual-Species Biofilms Reveals Species-Dependent Internal Organisation** - Connor MacDonald

**P6.14 - *Pseudomonas aeruginosa* drives growth and biofilm formation in obligate anaerobes from chronic wound biofilms** - Faizan Sadiq

**P6.15 - Photodynamic inactivation and farnesol as potential game changers in the elimination of frightening nosocomial combination: *Candida albicans* and *Enterococcus faecium* biofilms**  
Lucia Černáková

**P6.16 - The effects of epoxytiglane EBC-1013 on obligate anaerobic chronic wound bacteria**  
Jacob O. Evans

**P6.18 - Fungal-bacterial interactions in dual-species oral biofilms formed by *Candida albicans* and *Aggregatibacter actinomycetemcomitans*** - Adèle Huc

**P6.19 - In vitro evaluation of polymicrobial biofilms of *Staphylococcus aureus* and clinically relevant *Candida* spp for biocidal activity studies** - Alexia Gómez-Herrera

## **Session 8: Beneficial biofilms (microbiome)**

**P8.1 - Identifying biofilm-forming conditions in probiotic bacteria** - Alexander Brooks

## **Session 11: Host-pathogen interactions**

**P11.1 - Urinary secretory IgA and IgG responses and inflammatory markers in patients with biofilm forming *Pseudomonas aeruginosa* urinary tract infections** - Ayse Corap Cakmak

**P11.2 - Bone Microenvironment Promotes *Cutibacterium acnes* Adhesion and Biofilm Formation on Titanium** - Jennifer VARIN-SIMON

**P11.3 - *Staphylococcus aureus* forms surface-associated Z-DNA that promotes a potent proinflammatory response in THP-1 macrophages** - Freja Winther Sillesen

**P11.4 - Ex vivo nail model on the unraveling of host-pathogen interactions of mono and polymicrobial biofilms of *Trichophyton rubrum* on onychomycosis and involved genes**  
Bruna Almeida Furquim de Camargo

**P11.5 - Biofilm formation as a lineage-associated trait in invasive Extraintestinal Pathogenic *Escherichia coli*** - Elizabeth G Aarag Fredheim

**P11.6 - Stage-resolved analysis of mucosa-associated biofilms on electrospun host-mimetic scaffolds**  
Marie-Christine Rau

**P11.8 - Impact of *S. aureus* virulence on pathogenesis and host immune response in *G. mellonella* biofilm-mediated implant-associated infection models**  
Raphaëlle Youf

**P11.9 - Cells derived from biofilm formed in the pulmonary microenvironment exhibit differentiated virulence in the *Galleria mellonella* model.**  
Marcos William de Lima Gualque



**19 JUNE**  
Friday

**14:00 - 14:30**

**P11.10 - The importance of umoB gene in biofilm formation and host-pathogen interactions in *Proteus mirabilis*** - Wanesa Sasal

**P11.11 - Same Bacteria, Different Biofilm: Experimental influences on *Staphylococcus aureus* Biofilms** Safae Oukrich

**P11.12 - Infective Endocarditis Biofilms: Targeting the Foundation** - Kyo J. P. H. Renshof

**P11.13 - *Candida albicans* pulmonary infection in wildtype and Cystic Fibrosis-like mouse models highlight lipid dysregulation as a pathogenic mechanism** - Janette M Harro

**P11.14 - Antibiotic susceptibility and phage response of biofilm-producing *Enterococcus faecalis*** Sophio Rigvava

## **Session 12: Emerging control strategies**

**P12.1 - Natural Deep Eutectic Solvents as Alternative Agents for Biofilm Control.** Marwa Naguib

**P12.2 - Development of a Biofilm Drain Model and its Susceptibility to Treatment with Sodium Trocloses** Elizabeth O'Gorman

**P12.3 - Investigating the antibiofilm activity of free and encapsulated polyphenols in static and dynamic conditions** - Rossella Mauro

**P12.4 - Developing a Model System to Test Efficacy of Antimicrobial Materials for the Control of Biofilm-Forming Cyanobacteria** - Lauren Amy Jenkinson

**P12.6 - Antimicrobial and antibiofilm activity of ebselen-based inhibitors of bacterial ureases** Wojciech Tabor

**P12.7 - A Hydrogel-Based Microneedle System to Overcome Antibiotic-Resistant Wound Biofilms** Anna Hu

**P12.8 - EFFECTS OF L7CF197 ON BIOFILMS FORMED BY *Candidozyma auris*** - Débora Silva Marques de Sousa

**P12.9 - Exploring antifungal activity of polyphenolic and nitrofurantoin compounds on *Trichophyton rubrum* planktonic and biofilm forms: A tug of war against drug resistance** - Matheus Bordoy Mendonça

**P12.10 - Functional Fungal Proteins and Nanomaterials as Novel Strategies for Biofilm Control** Nika Zaveršek

**P12.11 - Combating implant-related orthopaedic infections with therapeutic ultrasound and shockwaves** Veerle Brans

**P12.12 - Advancing Biofilm Control: Functionalized-Nanomaterials for Light-Triggered Antibiofilm Action** Katarína Bilská



**19 JUNE**  
Friday

**14:00 - 14:30**

**P12.13 - Corticosteroids increased the production of Quorum Sensing Signalling Molecules in a biofilm-growing clinical *Pseudomonas aeruginosa* isolate from Chronic Obstructive Pulmonary Disease**  
Elena Jordana Lluh

**P12.14 - Nitrogen-Doped Graphene Polymeric Composites Inhibit Single- and Multi-Species Uropathogenic Biofilms** - Francisca Sousa-Cardoso

**P12.15 - Species-Specific Antibiofilm Activity of the Bacteriophage-Derived Endolysin CHAPk Against MRSA and MRSE** - Semra Tasdurmazli

**P12.16 - Evaluation of a Long-Lasting Disinfectant for Biofilm Control in Hospital Greywater Systems**  
Débora Castro

**P12.17 - Effects of gallic acid-based antifouling agents toward representative marine bacteria**  
Beatriz Freire

**P12.18 - ESKAPE-ing Antimicrobial Resistance; 213 nm laser light to combat superbugs**  
Harriet Cooling

**P12.19 - Dual-Layer Living Hydrogel Enables On-Demand Delivery of Phages and Probiotics for Synergistic Wound Infection Therapy** - Qun Ren

**P12.20 - Evaluating the disinfection efficacy of BDMDAC biocide in biofouling control** - Marta Redondo

**P12.21 - Identification of a bacteriophage that reduces the biofilm of a hypervirulent *Klebsiella pneumoniae* strain** - Sophia Hanstein

**P12.22 - Copper-Decorated Multi-Walled Carbon Nanotubes for the Control of Medical Device-Associated Biofilms** - Rita Teixeira-Santos

**P12.23 - Targeted Delivery of a Nucleoside Analog via a Lipoglycopeptide Conjugate to Eradicate Antibiotic-tolerant Persister Cells** - Nasiha Jahan Muna

**P12.24 - Antibiofilm and antipathogenic effects of a hydroethanolic extract of *Tanacetum vulgare*, a promising yet underexplored medicinal plant** - Veronica Lazar

**P12.25 - Crystal structure and antibiofilm activity determination of alterocin, a secreted protein from a marine bacterium** - Emma LE BORGNE

**P12.26 - Comparison of direct and indirect plasma treatment of common uropathogens** - Matthew McAteer

**P12.27 - Effect of the acyl-homoserine lactonase Aii20J on *Klebsiella pneumoniae* biofilm formation**  
Ana Otero

**P12.28 - Targeted biofilm disruption in bone infections using ultrasound-activated microbubbles for enhanced antibiotic delivery** - Aya Ben Issa

**19 JUNE**  
Friday

**14:00 - 14:30**

**P12.29 - Targeting Wound Biofilms with Cold Atmospheric Plasma via Direct Treatment and Dual-Therapy Strategies** - Jason L Brown

**P12.30 - Extending antisense nucleic acid delivery from planktonic cultures to biofilm models**  
Mariana Gomes

**P12.31 - Antibiofilm effect of blackthorn fruit extract on Staphylococcus aureus and Listeria monocytogenes** - Anna Draszanowska

**P12.32 - Localized Antibiotic-Release Coatings: Toward Antimicrobial Orthopedic Implants**  
Himanshu S. Mhaskar

**P12.33 - Phytochemical Quorum Sensing Inhibition as a Targeted Strategy Against Biofilm-Forming Multidrug-Resistant bacteria** - Boudhyayan Chatterjee

**P12.34 - Bio-Inspired Functionalized Nanocellulose Coatings for Biofilm Control** - Dina El Jaramany

**P12.35 - Synergistic Inhibition of Candida glabrata biofilms by Antisense Oligonucleotides Targeting PDR1 Combined with Fluconazole** - Patrícia Serra

**P12.36 - Development of a Novel Graft Coating for the Prevention of Vascular Graft Infections**  
Raoul Craeymeersch

**P12.37 - Looking into the human microbiome for new multifunctional peptides** - Maria Olivia Pereira

**P12.38 - Screening of natural natriuretic peptides dispersal activity on Pseudomonas aeruginosa biofilm in a dynamic flow-cell model** - Adrien Forge

**P12.39 - CRACKING PSEUDOMONAS AERUGINOSA BIOFILMS: SMALL-MOLECULE LIBRARY STRIKES BACK**  
Petra Seliškar

**P12.40 - Monobactams as double agents: killing bacteria and disarming biofilms** - Vid Kavaš

**P12.41 - Antimicrobial Functionalization of an Injectable Calcium Phosphate Cement for Prevention of Orthopaedic Implant-Associated Biofilm Infections** - Beatriz Marques

**P12.42 - Propolis-Loaded Chitosan Nanoparticles as a Sustainable Strategy for the Treatment of Mixed Paracoccidioides brasiliensis–Pseudomonas aeruginosa Biofilms** - Ana Marisa Fusco Almeida

**P12.43 - Effect of citric acid-based compounds with silver ions against biofilms formed by Listeria monocytogenes and Pseudomonas fluorescens isolated from the food industry** - Muhammad Tauseef Ahmad

**P12.44 - Bioactivity-Guided Fractionation of the Andean Plant Macleania rupestris Enhances its Anti-Biofilm Activity Against Staphylococcus aureus** - Arianna Mayorga-Ramos

**P12.45 - Antifungal activity of a new class of 2-aminoimidazole azo dyes on C. krusei and C. auris biofilms**  
Maria Inês Pacheco

**P12.46 - Polymeric nanoformulations encapsulating bioactive agents embedded in a hydrogel for multifunctional chronic wound healing**  
M. Machado

# PROGRAM



**19 JUNE**  
Friday

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|---------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 14:00 - 14:30 | <p><b>P12.47 - CLEC3A-Derived Antimicrobial Peptides as Novel Agents Against Fungal Biofilms: Mechanistic Insights and Clinical Implications</b> - Jens Beckers</p> <p><b>P12.48 - Cross-Kingdom Communication: Effects of Giardia lamblia Extracellular Vesicles on Bacterial Biofilms</b> - Maria Céu Sousa</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| 14:30 - 16:00 | <p style="text-align: right;"><b>Auditorium A</b></p> <p><b>Session 10</b><br/><b>Biofilm tolerance and resistance</b></p> <p><b>Moderators:</b> Olívia Pereira and Tom Coenye<br/><b>Invited Speaker:</b> Dirk Bumann<br/>Understanding antimicrobial treatment failure in human staphylococcus aureus infections</p> <p>Selected Oral Presentations</p> <p><b>OP10.1 - Response of Resistant Candida albicans Biofilms to PDI-Induced Oxidative Stress</b><br/>Helena Bujdáková</p> <p><b>OP10.2 - Investigating the effects of disinfectants against dry and hydrated Candidozyma auris biofilms</b><br/>Gordon Ramage</p> <p><b>OP10.3 - Within-host adaptation during relapsing infective endocarditis selects for increased bacterial resilience</b> - Øyvind Myrvoll Lorentzen</p> <p><b>OP10.4 - Contribution of the biofilm extracellular matrix to Salmonella gene regulation and oxidative stress responses</b> - Leire Azparren</p> |
| 16:00 - 16:30 | <p><b>Coffee Break</b></p> <p style="text-align: right;"><b>Balcony of 2nd floor</b></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 16:00 - 18:00 | <p style="text-align: right;"><b>Auditorium A</b></p> <p><b>Session 11</b><br/><b>Host-pathogen interaction</b></p> <p><b>Moderators:</b> Dirk Bumann and Mariana Henriques<br/><b>Invited Speaker:</b> Céu Figueiredo<br/>Beyond helicobacter pylori: the emerging role of the gastric microbiome in gastric cancer</p> <p><b>Selected Oral Presentations:</b></p> <p><b>OP11.1 - Adaptive Trajectories to Ciprofloxacin Resistance in a Murine Biofilm Lung Infection Model</b><br/>Doaa Higazy</p> <p><b>OP11.2 - Sorbed host proteins mediate neutrophil adhesion, motility, and discovery of Staphylococcus aureus on an abiotic surface</b> - Philip S. Stewart</p>                                                                                                                                                                                                                                                                       |

# PROGRAM

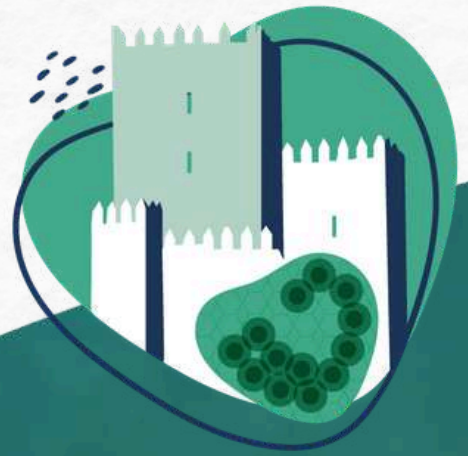


**19 JUNE**  
Friday

|                      |                                                                                                                                                                                                                                                                                                                                                                                       |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>16:00 - 18:00</b> | <p><b>OP11.3 - Establishment of a chronic orthopaedic infection model with mature biofilm formation using an in vitro-in vivo hybrid approach</b> - <a href="#">Raphaëlle Youf</a></p> <p><b>OP11.4 - The putative AHL receptor SdiA as a repressor of biofilm formation, phagocytosis and intracellular survival in Klebsiella pneumoniae</b> - <a href="#">Sergio Silva-Bea</a></p> |
| <b>18:30</b>         | <b>Guided Walking Tour - Guimarães City Center</b>                                                                                                                                                                                                                                                                                                                                    |

**20 JUNE**  
Saturday

|                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                      | <b>Auditorium A</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>09:00 - 10:30</b> | <p><b>Session 12</b><br/><b>Emerging control strategies</b></p> <p><b>Moderators:</b> Holger Rohde and Maria Dolores Macia Romero<br/><b>Invited Speaker:</b> <b>Tristan Ferry</b><br/>Phage therapy for biofilm-associated infections: real-world experience from 100 patients treated in france through the “phageinlyon clinic” programme</p> <p>Selected Oral Presentations</p> <p><b>OP12.1 - A Wearable Intracorneal Metalloenzyme-Microneedle Platform Embedded with an Inbuilt Antibacterial Function through Inorganic DNase for the Treatment of Keratitis Infections</b> - Sumanta Ghosh</p> <p><b>OP12.2 - N-terminal lipidation boosts antifungal lipopeptides with activity against mature Candida biofilms and improved membrane targeting</b> - Damiano Squitieri</p> <p><b>OP12.3 - Defying Gravity: Cold Plasma Technology to Defeat Biofilms in Space</b> - Georgina Neville</p> <p><b>OP12.4 - Targeted Phage Therapy Effectively Disrupts Klebsiella pneumoniae Biofilms: Implications for Wound Infection Treatment</b> - Srikanth Ravishankar</p> |
| <b>10:30 - 11:00</b> | <p><b>Coffee break</b></p> <p><b>Balcony of 2nd floor</b></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>11:00 - 12:00</b> | <p><b>Auditorium A</b></p> <p><b>Keynote 2: Unraveling periprosthetic joint infection through a biofilm lens</b></p> <p><b>Invited Speaker: Robin Patel</b></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| <b>12:00 - 12:30</b> | <p><b>Awards &amp; Closing Session</b></p> <p><b>Auditorium A</b></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |



# 7

Abstracts  
SPEAKERS



## MICROBIOME ECOLOGY FOR CANCER CARE

**Joao Xavier (United States)<sup>1</sup>**

1 - Memorial Sloan Kettering Cancer Center

Cancer therapy perturbs the gut microbiome in ways associated with worse clinical outcomes, including impaired response to immunotherapy, opportunistic infections, and graft-versus-host disease. Despite this strong clinical evidence, oncology practice often neglects the microbiome, in part because of its biological complexity. I propose an ecological framework to operationalize the microbiome for cancer care. Ecology reframes treatment-induced disruption as transitions between measurable ecosystem states characterized by biodiversity loss, functional impairment, and susceptibility to domination by opportunistic pathogens. I will describe our longitudinal studies in patients undergoing allogeneic hematopoietic cell transplantation, which demonstrate that reduced microbiome biodiversity and pathogen-dominated states can lead to infections, slower immune recovery, and worse patient survival. Importantly, low diversity is not a uniform condition but encompasses distinct ecological states with different clinical implications.

The ecological view of the microbiome enables three clinically actionable strategies: (i) monitoring, through serial microbiome profiling to detect high-risk ecological states; (ii) preservation, by incorporating microbiome impact into therapeutic decision-making and drug stewardship; and (iii) repair, using interventions such as autologous fecal microbiota transplantation or rational microbial consortia, guided by ecological principles such as niche availability and ecosystem receptivity. Clinical trials support the feasibility of modulating the microbiome to improve cancer treatment outcomes.



## **PRECISION DIAGNOSTICS IN PROSTHETIC JOINT INFECTIONS: EMERGING TECHNOLOGIES AND STRATEGIES**

**Holger Rohde (Germany)<sup>1</sup>**

1 - University Medical center Hamburg-Eppendorf

Prosthetic joint infections (PJI) represent a growing clinical and socioeconomic burden as the number of joint arthroplasties continues to rise worldwide. Despite increasing clinical demand for rapid and reliable diagnostics, the diagnosis of PJI remains challenging. Two major objectives are central to the diagnostic workup: accurate identification of infection and reliable detection of the causative pathogen. However, no single standalone assay currently enables an unequivocal diagnosis.

Conventional inflammatory parameters derived from synovial fluid analysis can support clinical decision-making, yet their diagnostic sensitivity and specificity remain limited, particularly in low-grade infections and complex clinical scenarios. Recent advances in the understanding of host inflammatory responses have facilitated the identification of novel biomarkers with the potential to improve diagnostic accuracy. In parallel, culture-independent molecular technologies, including next-generation sequencing (NGS), are increasingly being introduced for pathogen detection from synovial fluid and periprosthetic tissue biopsies.

This talk will provide an overview of the rationale underlying these emerging diagnostic developments and discuss current evidence supporting their clinical implementation. Particular emphasis will be placed on the opportunities and limitations associated with NGS-based approaches, including diagnostic sensitivity, contamination challenges, interpretation of complex datasets, and integration into routine clinical workflows. The presentation aims to highlight how precision diagnostic strategies may shape the future management of prosthetic joint infections.



## EXPLORING BIOFILM FORMATION IN THE GUT SYMBIONT BACTEROIDES THETAOTAOMICRON

1 - Institute Pasteur

**GHIGO Jean-Marc (France)**

Despite the medical, environmental, and industrial relevance of bacterial biofilms, this widespread lifestyle has been studied primarily in aerobic bacterial species. We investigated biofilm formation in *Bacteroides thetaiotaomicron*, an anaerobic gut symbiont that degrades dietary polysaccharides and contributes to intestinal maturation. Although the ability of *B. thetaiotaomicron* to adhere to host tissues, bacterial surfaces, and food particles may play an important role in gut colonization and homeostasis, little is known about the factors and conditions that promote its biofilm formation. I will present recent work combining genetic and biochemical approaches to identify the surface structures and environmental conditions involved in *B. thetaiotaomicron* adhesion and biofilm development. These findings provide new insights into the role of biofilm formation by gut symbionts in shaping the stability and functional dynamics of the intestinal microbiota.



## **INNOVATING THE BIOFILM TOOLBOX: SYNTHETIC *IN VIVO*-LIKE MEDIA MEET ADVANCED ANALYTICS**

**Tom Coenye (Belgium)<sup>1</sup>**

1 - Ghent University

In this presentation I will discuss the added value of using synthetic *in vivo*-like media to study multiple aspects of biofilms (including virulence and antimicrobial susceptibility) in various contexts, including the CF lung, prosthetic joint infections and chronic wound infections. In addition, I will highlight the potential of isothermal microcalorimetry to learn more about biofilm physiology and discuss its potential for rapid diagnosis of biofilm-related infections.



## **SINGLE-CELL IMAGING OF MICROBIAL COMMUNITIES ACROSS DIFFERENT SPATIAL AND TEMPORAL SCALES**

**Jing Yan (United States)<sup>1</sup>**

1 - Yale University

Biofilm is an important lifestyle of bacteria where bacterial cells collectively form surface-associated aggregates embedded in a polymeric matrix they secrete. Important in industry and medicine, biofilms also represent a unique form of growing active matter with unique physical and material properties. In this talk, I will highlight our recent work on developing a platform for imaging live, growing biofilm at single-cell resolution, using *Vibrio cholerae* as the model organism. I will show how bacterial cells physically communicate with each other and the environment to form ordered architecture reminiscent of liquid crystals. Moreover, I will talk about our recent discovery that heterogeneity in gene expression leads to unexpected pattern formation in biofilm, and how we used agent-based simulations to reveal the drag-based sorting mechanism underlying the observation. I will also highlight ongoing research in the lab on extending the imaging capability to more natural environments including animal hosts.



## ORAL BIOFILMS IN HEALTH (OBIH-1)

**Bastiaan P. Krom (Netherlands)**

1 - Academic Centre for Dentistry Amsterdam (ACTA)

Oral microbial biofilms are central to oral and systemic health during all phases of life. They cause two of the most prevalent diseases in humans worldwide, dental caries and periodontitis. Oral biofilms are also implicated in systemic diseases such as diabetes, rheumatoid arthritis and neurodegenerative diseases (*e.g.* Alzheimer's and Parkinson's disease).

Oral biofilms are highly dynamic in time and space and are impacted by external factors including the host (age, sex, genetics, diseases) and the environment (diet, smoking, oral hygiene, medications including antibiotics). This dependency on cultural-behavioural factors provide unique opportunities for population- based interventions supporting prolonged vitality for an aging population. Research aimed at care (prevention) and cure (treatment) of oral and systemic diseases will impact individuals globally and improve preservation of health and maintain the quality of life in diverse life-stages.

In light of this enormous importance of oral biofilm research and future promises in oral and general healthcare, the Oral Biofilms in Health (OBIH-1) COST-action initiative is announced. This envisioned COST-action aims to bring together a diverse consortium of researchers from different disciplines studying different aspects related to oral biofilms through different means.

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## **BEHAVIOUR AND ADAPTATION IN MULTISPECIES BIOFILMS ARE DEFINED BY NEIGHBOURS**

**Henriette Lyng Røder (Denmark)<sup>1</sup>**

1 - Section for Food Microbiology, Gut Health and Fermentation, University of Copenhagen

We still cannot reliably predict how microbial communities behave, despite their central roles in health, agriculture, industry, and the environment. While significant progress has been made, key aspects remain unresolved, and as a result, we often fail to control these systems.

In my work, combining ecological and evolutionary theory with defined experimental systems, reveals a consistent pattern: in multispecies biofilms, behaviour depends on interactions between neighbouring populations. Across systems, the same strains follow different trajectories in biofilm formation, matrix production, and persistence, depending on community composition. Biofilms may either form, reorganise, or completely fail to develop under otherwise comparable conditions. These differing outcomes cannot be explained from individual organisms alone, and are consistently observed across independent experimental setups. This becomes particularly evident in structured environments, where biofilms develop within defined matrices. In various systems, including recently developed mucus-associated gut model systems, bacteriophage-driven changes alter biofilm structure and community organisation without substantially changing overall composition. Functional outputs, including matrix production, and stability, reflect interactions between neighbours as a result of the specific composition.

This also shapes adaptation, as evolutionary changes occur in the presence of neighbouring populations and repeatedly converge on specific phenotypes. In our systems, this includes reduced matrix production and strategies that promote coexistence rather than competitive exclusion, suggesting constrained adaptive pathways. These outcomes emerge within spatially structured biofilms, where local interactions and horizontal gene transfer redistribute traits and constrain which phenotypes persist.

These evidences clearly point towards that biofilm behaviour and adaptation are defined by interactions between neighbours. If we do not understand the neighbours, we cannot predict, or hope to control, what biofilms actually do.



## **BENEFICIAL BIOFILMS - A FOCUS ON THE EARLY LIFE GUT MICROBIOTA**

**Lindsay Hall (UK)<sup>1</sup>**

1 - University of Birmingham

Early life is a defining period for microbiome assembly, when infant gut communities behave as structured, surface-associated ecosystems shaped by colonisation dynamics, metabolite exchange, and competitive exclusion. In this talk, I will discuss beneficial *Bifidobacterium* as ecological “foundation species” that can guide these early-life communities toward stability in preterm and term infants. I will summarise evidence from our studies linking targeted supplementation to reduced serious infections in vulnerable newborns, and the mechanisms that underlie these effects including how colonising strains and their metabolites reshape microbe-microbe interactions, resource niches, and host interfaces to limit pathogen expansion and strengthen community resilience, including recent findings indicating reduced antimicrobial-resistance gene carriage. Finally, I will discuss how broader community interactions drive favourable ecological transitions across infancy and how these insights can guide precision microbiome interventions from the very start of life.



## UNDERSTANDING ANTIMICROBIAL TREATMENT FAILURE IN HUMAN STAPHYLOCOCCUS AUREUS INFECTIONS

Dirk Bumann (Switzerland)<sup>1</sup>

1 - Biozentrum, University of Basel

Deep-seated *S. aureus* infections are difficult to treat. Even after surgical debridement and extensive antimicrobial treatment, relapses remain common. Treatment failure has been attributed to aggregate/biofilm formation, intracellular residence, stress-induced tolerance, persister formation, and insufficient local antibiotic exposure, but most evidence comes from in vitro studies or animal models. The relevance of these mechanisms in human infections remain unclear because detection and quantification of *S. aureus* in human tissues is challenging due to sparse and heterogeneous distribution of the bacteria and high tissue autofluorescence. Using advanced imaging, we addressed these challenges enabling us to map ~41,000 *Staphylococcus aureus* cells in biopsies from 43 patients with musculoskeletal infections. A majority of bacteria resided within non-classical monocytes/macrophages. Intra- and extracellular bacteria were predominantly isolated single cells with low rRNA content, suggesting limited replication. In some biopsies, we also observed aggregates of thousands of bacteria. Biopsy proteomics and tissue-mimicking laboratory cultures revealed that ischemia-associated hypoxia and glucose scarcity restricted *S. aureus* growth. Antibiotic treatment prior to surgery did not reduce bacterial density or alter the intra-/extracellular ratio, host- cell tropism, or the proportion of aggregates, indicating broad antibiotic tolerance across diverse bacterial subsets. Our findings redefine *S. aureus* infection biology, identify bacterial starvation as main mechanism of antibiotic treatment failures, and highlight the importance of measuring pathogen physiology and host microenvironments directly in human tissues.



## BEYOND HELICOBACTER PYLORI: THE EMERGING ROLE OF THE GASTRIC MICROBIOME IN GASTRIC CANCER

Ceu Figueiredo (Portugal)<sup>1,2</sup>

1 - i3S - Institute for Research and Innovation in Health, University of Porto; 2 - Faculty of Medicine, University of Porto

Gastric cancer remains one of the most common and lethal malignancies worldwide. Although *Helicobacter pylori* is the major infectious trigger, only a fraction of infected individuals develop cancer. In recent years, microbiome research has offered a new perspective on the complexity of the microbial communities of the stomach, highlighting the emerging role of the gastric microbiome beyond *H. pylori* in gastric carcinogenesis. This presentation will discuss current knowledge and our recent findings on the gastric microbiome in the context of gastric cancer. A better understanding of these host-microbial interactions may open new opportunities for the prevention, diagnosis and treatment of patients with precancerous gastric disease, although further studies are still needed to clarify their clinical impact.



## PHAGE THERAPY FOR BIOFILM-ASSOCIATED INFECTIONS: REAL- WORLD EXPERIENCE FROM 100 PATIENTS TREATED IN FRANCE THROUGH THE “PHAGEinLYON CLINIC” PROGRAMME

**Tristan Ferry (France)**<sup>1</sup>; Cécile Batailler (France)<sup>1</sup>; Tiphaine Roussel-Gaillard (France)<sup>1</sup>; Myrtille Le Bouar (France)<sup>1</sup>; Pauline Lacroix (France)<sup>1</sup>; Ani Horikian (France)<sup>1</sup>; Florent Valour (France)<sup>1</sup>; Sylvain Goutelle (France)<sup>1</sup>; Sandrine Roux (France)<sup>1</sup>; Anne Conrad (France)<sup>1</sup>; Isabelle Eberl (France)<sup>1</sup>; Thomas Perpoint (France)<sup>1</sup>; Fabien Craighero (France)<sup>1</sup>; Sébastien Lustig (France)<sup>1</sup>; Thomas Briot (France)<sup>1</sup>; on behalf of the PHAGEinLYON Clinic programme (France)<sup>1</sup>

1 - Hospices Civils de Lyon

Biofilm-associated infections remain a major therapeutic challenge, as antibiotics alone frequently fail to eradicate bacteria embedded within biofilms, leading to persistent infections, repeated relapses, chronic inflammation, repeated surgical procedures, and potentially death. Very few antibiotics have demonstrated clinical benefit in these settings, notably rifampin against *Staphylococcus aureus* and fluoroquinolones against Gram-negative bacteria, while antimicrobial resistance increasingly limits their use. Bacteriophages (phages) are naturally occurring viruses that specifically infect and kill bacteria, generally with a narrow bacterial host range. The isolation and purification of naturally virulent phages targeting pathogenic bacteria have opened new perspectives for the treatment of biofilm-associated infections, as phages may also act directly on biofilms through various mechanisms. We report the largest real-world clinical experience of phage therapy in Europe, based on 100 patients treated within the French “PHAGEinLYON Clinic” programme under compassionate use and supervision of the French healthcare authorities. Most patients presented with severe osteoarticular, cardiovascular implant-associated, or pulmonary biofilm-associated infections. Most were infected with *Staphylococcus aureus* or *Pseudomonas aeruginosa*, two major virulent pathogens and strong biofilm producers associated with poor clinical outcomes. Phages were mainly provided by a French company (PP1493 and/or PP1815 targeting *S. aureus*; PP1450, PP1777, PP1792 and/or PP1797 targeting *P. aeruginosa*) and few from a Military Hospital. Patients were managed through dedicated multidisciplinary phage therapy meetings integrating infectious disease specialists, microbiologists, surgeons, pharmacists, and pharmacologists. Preliminary results support phage therapy as a valuable adjunctive option with encouraging clinical improvement and a favourable safety profile. Among patients treated during 2022, 69% showed favourable clinical evolution despite highly complex situations frequently associated with severe functional impairment or life-threatening prognosis. Some nevertheless required subsequent phage therapy with prolonged exposure or newly selected phages, suggesting that optimization and individualization of phage exposure may further improve outcomes.

Major challenges remain, including improved understanding of phage-biofilm interactions, host immune responses and pharmacokinetics, development of new formulations, and the conduct of clinical trials using methodologies specifically adapted to phage therapy. By bridging compassionate use and clinical research, the “PHAGEinLYON Clinic” programme represents a pioneering translational model for integrating phage therapy into modern healthcare systems.



## UNRAVELING PERIPROSTHETIC JOINT INFECTION THROUGH A BIOFILMS LENS

**Robin Patel (United States)<sup>1</sup>**

1 - Mayo Clinic

Arthroplasty-associated infection, or periprosthetic joint infection (PJI), is a relatively rare but increasingly recognized complication of joint replacement surgery that involves interactions between microorganisms, implanted prosthetic material, and the host immune system. A small inoculum of microorganism can be sufficient to establish infection, as pathogens readily adhere to implant surfaces and form biofilms, which protect them from host defenses and many antimicrobial agents. Causative microorganisms are commonly members of the skin microbiota, oftentimes introduced at implant placement. A wide range of bacteria—and, much less commonly, fungi—cause PJI. The most frequently implicated microorganisms include coagulase-negative staphylococci (particularly *Staphylococcus epidermidis*), *Staphylococcus aureus*, *Streptococcus* species, *Enterococcus* species (especially *Enterococcus faecalis*), *Cutibacterium acnes*, *Pseudomonas aeruginosa*, and members of the *Enterobacteriaceae*. Culture-negative PJI is a significant challenge. In the United States, the incidence of hip and knee PJI is about 2%. PJI is associated with substantial healthcare cost and has profound physical, social, and emotional impact on affected patients. Accurate diagnosis is critical, as management strategies differ from those used for noninfectious arthroplasty failure. When PJI is present, identification of microbial etiology is essential to guide surgical decision-making and antimicrobial therapy. Our team has developed multiple diagnostic approaches, including novel biofilm-sampling strategies, to improve PJI diagnosis. We have also conducted advanced deep nucleic acid sequencing and proteomic analyses to characterize both PJI and noninfectious arthroplasty failure. Treatment of PJI is complex, resource-intensive, and often prolonged. Antibiotic therapy alone is typically insufficient; effective management classically requires combined surgical intervention and antimicrobial treatment. An overview of novel therapeutic strategies for PJI evaluated by our team will be presented.



## LASER-ASSISTED BIOPRINTING TO CHARACTERIZE COMPLEX PATHOGENIC BIOFILMS

Szymon P. Szafranski (Germany)<sup>1 2 3</sup>

1 - Hannover Medical School

1 - Department of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School, Hannover, Germany; 2 - Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (NIFE), Hannover, Germany; 3 - Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, Hannover, Germany.

(i) Background & Aims. Oral peri-implant biofilms frequently drive recalcitrant infections that require timely, tailored antimicrobial therapies. However, the high complexity of these microbial communities hampers accurate assessment of biofilm virulence, synergistic interspecies interactions, and antimicrobial tolerance. Although metagenomics has revolutionized microbiome profiling, mechanistic investigation remains constrained by the lack of comprehensive and representative culture collections. This project aims to develop a technology platform that accelerates, miniaturizes, and spatially structures microbial culturomics workflows. (ii) Methods. A laser-assisted culturomics platform is developed, that integrates bioprinting, optimization of culture conditions, microbial characterization, and biobanking. Laser-assisted bioprinting enables rapid and precise transfer of microorganisms from complex clinical biofilms into highly ordered colony arrays that are suitable for co-culture and molecular analyses. (iii) Results. Using this approach, 99% of tested species were successfully propagated, and 79% of abundant dental plaque species were recovered. Application of this technology to oral biofilms from four individuals yielded 124 species spanning 14 microbial classes. Functional profiling identified commensal species with potential roles in disease development. Isolates from peri-implantitis samples represented 85.4% of transcriptionally active genera in clinical biofilms. Furthermore, we developed a laser-assisted antimicrobial susceptibility testing strategy that uncovered collective tolerance to amoxicillin in refractory peri-implantitis biofilms. (iv) Conclusions. Overall, this platform enables on-demand generation of culture collections and engineered biofilms for both research and clinical applications. Importantly, our findings provide a mechanistic framework for polymicrobial tolerance and highlight potential diagnostic and therapeutic targets for implant-associated oral biofilm infections.

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## HORMONES AS HOST-DERIVED MODULATORS OF BIOFILMS

**Aurea Simon-Soro (Spain)<sup>1 2</sup>**

1 - 1 - Department of Stomatology, University of Seville, 41009 Sevilla, Spain; 2 - Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER) Sevilla Spain.

Biofilm communities are shaped by their physicochemical environment yet the endocrine signals continuously present in mucosal fluids have received little attention as ecological drivers. Steroid hormones circulate in saliva and gingival crevicular fluid at physiologically relevant concentrations, fluctuating across the lifespan and in response to disease. Whether oral biofilms sense, respond to, and actively participate in hormonal metabolism remains an open question with consequences for how we model host-biofilm interactions. The relationship is bidirectional and context-dependent: the same hormonal signal produces qualitatively distinct biofilm responses depending on whether exposure is chronic, pathological, or acute. Critically, certain oral taxa appear not as passive recipients of hormonal cues but as active participants in local hormone metabolism, a microbial capability that may feed back on the very signals regulating biofilm ecology.

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## HACKING ORAL BIOFILMS WITH SMART NANOTECHNOLOGIES AND MICROROBOTS

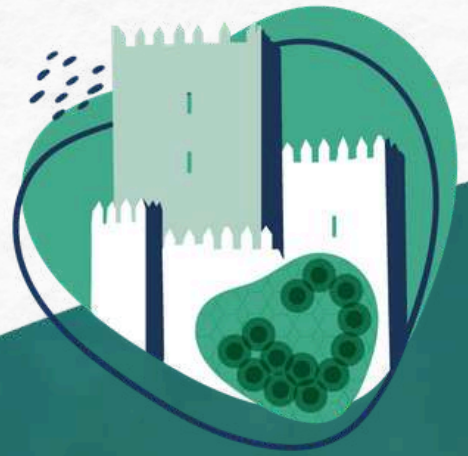
Hyun (Michel) Koo (United States)<sup>1,2</sup>

1 - School of Dental Medicine and School of Engineering; 2 - University of Pennsylvania

Oral biofilms are spatially organized, matrix-protected microbial communities whose pathogenic behavior emerges from interspecies interactions, local chemistry, host-derived cues, and difficult-to-access microenvironments. Conventional antimicrobial approaches often act broadly and passively, limiting their ability to interrogate or selectively disrupt biofilm structure and function. Our work integrates microbiology, catalytic nanomaterials, and microrobotics to develop precision tools for probing and reprogramming oral biofilms. Iron oxide nanozymes activate localized antimicrobial chemistry within diseased biofilm microenvironments, disrupting extracellular matrix and killing embedded pathogens while minimizing ecological disruption and preserving host-compatible repair responses. Building on this platform, magnetically guided microrobotic systems add motion, physical contact, and spatial control, enabling targeted delivery, localized catalysis, and mechanochemical biofilm disruption in confined niches. Together, these approaches move biofilm control beyond bulk antimicrobial treatment or physical removal toward tunable, spatially resolved interventions that can reveal, disrupt, and potentially reprogram pathogenic biofilm states.

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# 8

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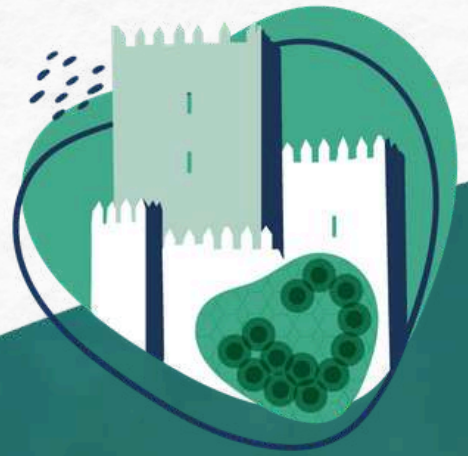
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9

Oral  
PRESENTATIONS



## OP1.1 - IDENTIFICATION OF GENES INVOLVED IN AGGREGATE ASSOCIATED ANTIMICROBIAL TOLERANCE OF *PSEUDOMONAS AERUGINOSA* USING TN-SEQ GUIDED DISCOVERY

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**Background & Aims:** It is estimated that 60-70% of hospital-acquired infections are caused by biofilms. Since current treatment regimens in many cases fail to cure biofilm-based infections, it is critical to identify the mechanisms which underlie biofilm-based antimicrobial tolerance. The aim of this study was to develop a transposon sequencing (Tn-Seq) based procedure that enables simultaneous assessment of the relative antibiotic tolerance of hundreds of thousands of individual aggregates, each established by a single transposon mutant; in order to obtain knowledge about the mechanistic basis of biofilm-associated antibiotic tolerance.

**Methods:** We used a *P. aeruginosa* PA14 transposon (Tn) mutant library to identify mutants with increased susceptibility towards a panel of antibiotics. The transposon mutants were grown in agar plates under conditions where each mutant formed an agar-embedded aggregate. The aggregates were treated with the antibiotic of choice or a saline control and surviving bacteria were released from the agar and their DNA extracted. The identity and relative number of the mutants present in the pool of antibiotic-treated and non- treated bacteria were then determined by Tn-Seq analysis.

**Results:** Antimicrobial tolerance increases with aggregate age but even relatively young aggregates display tolerance up to 10-fold higher than their planktonic counterparts. Furthermore, the level of c-di-GMP – as measured through a c-di-GMP-monitor – is significantly higher in the aggregates compared to planktonic growth, confirming that the aggregates represent a biofilm-like growth physiology. During antibiotic treatment, Tn- mutants with higher susceptibility were selectively depleted from the antibiotic-treated pool. Subsequently, all identified genes were ordered according to their potential importance for aggregate-associated antimicrobial tolerance thus serving as the starting point for exploring the underlying mechanisms.

**Conclusions:** We have developed a Tn-Seq based procedure that enables simultaneous assessment of the relative antibiotic tolerance of hundreds of thousands of individual bacterial aggregates. Using this genome-wide Tn- Seq analysis approach, we found that antimicrobial tolerance of *P. aeruginosa* aggregates depends on numerous different genes. Using our model, we can continue to expand on these datasets using different selection pressures such as antibiotics or growth conditions. This allows us to identify which genes are important for specific treatments and which are of general importance to the aggregate mode-of-growth.



### OP1.3 - ANTIMICROBIAL RESISTANCE PROFILING AND BIOFILM DETECTION IN CYSTIC FIBROSIS SPUTUM USING MX-RAMAN

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**Background & Aims:** A key challenge in biofilm research is to study their native host context without perturbation, as existing approaches rely on destructive labelling, hybridisation, or genetic reporters that are unsuitable for in-situ or in- vivo studies. Here we demonstrate an ability to carry out pathogen AMR profiling, and examine biofilm directly within clinical samples, using our Raman spectroscopy methodology, MX-Raman. We examine bacterial respiratory infections, which are primarily diagnosed using culture techniques and can take days to reach diagnosis. Lung infections typically exist as biofilm aggregates within mucus, which drive antimicrobial resistance (AMR) development and emphasise the need for rapid and effective treatment decisions.

**Methods:** MX-Raman combined data outputs from Raman spectroscopy analyses using excitation with laser wavelengths 532nm and 785nm. Twenty *Pseudomonas aeruginosa* strains were analysed using MX-Raman. Five additional *P. aeruginosa* strains were isolated from clinical sputum samples from cystic fibrosis patients, using *Pseudomonas* isolation agar. Microbial community analyses of the sputum samples were conducted using 16S sequencing. The AMR profiles of the strains were tested in accordance with EUCAST guidelines for antibiotics ceftazidime, ciprofloxacin, imipenem, and tobramycin. Nine classifiers were tested to assess the most effective classification model for the data, resulting in the use of support vector machine (SVM) analysis to predict AMR profiles of clinical *P. aeruginosa* isolates using their Raman spectra. Pearson's correlation coefficient (PCC) was used to calculate similarities between spectra from sputum samples and spectra from a respiratory pathogen library.

**Results:** SVM classified and identified the twenty *P. aeruginosa* strains with 93% accuracy. AMR profiles were predicted using SVM with an accuracy range of 91-96% across the four antibiotics. Sputum samples analysed with PCC correctly identifies the presence of *P. aeruginosa*, confirmed by microbiome analysis, demonstrating the ability of MX-Raman to detect pathogens for infection diagnosis directly from clinical samples.

**Conclusions:** These data provide evidence that MX-Raman can detect and characterise AMR profiles for respiratory pathogens, and has the potential to diagnose infection directly from biofilm within sputum samples. With further development, this technology could provide new insight into host-biofilm interactions, be a rapid near- to-patient solution for infection diagnosis, and could reduce the spread of AMR.



## OP1.4 - MRNA PNA-FISH AS A SPATIAL TRANSCRIPTOMICS PLATFORM FOR SINGLE-CELL GENE EXPRESSION ANALYSIS IN BIOFILMS

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**Background & Aims:** Biofilms are inherently heterogeneous structures in which localized chemical gradients drive physiological diversification among genetically similar cells. Within these communities, cells adopt spatially distinct physiological states, displaying division of labor, varied metabolic activities and differential stress responses. Understanding biofilm organization at both community and single-cell levels is essential to uncover mechanisms underlying microbial persistence and tolerance. Such knowledge is critical for improving strategies to control or eradicate biofilms, with implications for public health. While techniques as qPCR and scRNA-seq have significantly advanced our understanding of microbial gene expression, maintaining spatial context remains a major challenge. Spatial transcriptomics approaches overcome this limitation by preserving biofilm architecture while enabling spatially resolved gene expression analysis. In here, I introduce a robust, non-disruptive spatial transcriptomics platform based on mRNA Peptide Nucleic Acid Fluorescence in situ Hybridization (mRNA PNA-FISH). Designed for direct in situ visualization and quantification of mRNA, this method enables spatial mapping of regulatory networks across diverse microbial species and complex biofilm communities.

**Methods:** Gene-specific PNA probes targeting selected mRNA transcripts of *Legionella pneumophila*, *Listeria monocytogenes* and wound-associated species were designed and fluorescently labelled. Owing to their neutral peptide backbone, PNA probes exhibit strong and specific hybridization to complementary RNA and high resistance to enzymatic degradation. Hybridization protocols were optimized for both planktonic cells and intact biofilms to enhance probe penetration while preserving structural integrity. Biofilms grown under controlled conditions were fixed and analyzed using mRNA PNA-FISH, with independent validation by RT- qPCR. Confocal laser scanning microscopy enabled three-dimensional visualization of transcript distribution, and quantitative image analysis assessed spatial expression patterns and relative transcript abundance.

**Results:** Multiplex mRNA PNA-FISH assays enabled robust detection of target transcripts within intact biofilms. High signal intensity and low background fluorescence allowed single-cell resolution imaging across distinct biofilm niches, revealing marked cellular heterogeneity. The method demonstrated high specificity, absence of cross-reactivity, and reproducibility across independent experiments.

**Conclusions:** mRNA PNA-FISH is a sensitive and reliable method for spatial gene expression analysis in biofilms, enabling detailed characterization of microbial heterogeneity and supporting research into biofilm physiology, antimicrobial resistance, and pathogenic mechanisms.



## OP2.1 - ANTIPARALLEL STACKING OF CSU PILI DRIVES ACINETOBACTER BAUMANNII 3D BIOFILM ASSEMBLY

Anton Zavialov (Finland)<sup>1</sup>

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**Background & Aims:** Many Gram-negative nosocomial pathogens rely on adhesive filaments, known as archaic chaperone-usher pili, to establish stress- and drug-resistant, multi-layered biofilms. Here, we uncover the mechanism by which these pili build three-dimensional (3D) biofilm architectures.

**Methods:** We applied a combination of scanning, negative stain and cryogenic electron microscopy to study *A. baumannii* biofilms and isolated networks of Csu pili.

**Results:** In situ analyses of *Acinetobacter baumannii* biofilms using electron microscopy (EM) reveal an extensive network of ultrathin, flat stacks of archaic Csu pili interconnecting bacterial cells in 3D space. Cryo-EM structures of a single native pilus, pilus pairs, and two types of multi-pilus stacks show that the pili pack into antiparallel sheets, with their rods connected laterally by junctions at their zigzag corners. This antiparallel arrangement ensures that contacts form primarily between pili from interacting cells rather than pili from the same cell. With a remarkably short helical repeat, archaic chaperone-usher pili spontaneously establish a high density of junctions that determines the biofilm's 3D architecture.

**Conclusions:** We reveal that Csu pilus rods organize into flat antiparallel sheets linking neighbouring cells in three-dimensional space. Meanwhile, poly-N-acetylglucosamine (PNAG) fills the gaps within the Csu stack networks, collectively providing protection to the bacteria. Our findings may help develop new therapies against multidrug-resistant bacterial infections by targeting pilus-pilus interactions. Reference: Henri Malmi, Natalia Pakharukova, Bindusmita Paul, Minna Tuittila, Irfan Ahmad, Stefan David Knight, Bernt Eric Uhlin, Debnath Ghosal and Anton Zavialov. Antiparallel stacking of Csu pili drives *Acinetobacter baumannii* 3D biofilm assembly. *Nature Communications*, in press.



## OP2.2 - FUNCTION AND SPATIO-TEMPORAL PATTERNING OF THE SMALL BASIC PROTEIN (SBP) IN STAPHYLOCOCCUS EPIDERMIDIS BIOFILM FORMATION

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**Background & Aims:** *Staphylococcus epidermidis* is a common nosocomial pathogen causing implant-related infections. Pathogenicity essentially relies on biofilm formation, driven by the production of an extracellular matrix. The extracellular matrix provides structural stability, adhesion, and spatial heterogeneity, yet its molecular organization and functional interactions remain poorly understood. Previously, an 18 kDa protein, named small basic protein (Sbp), was identified as a dominant biofilm matrix component. This study aimed to characterize the interactions and temporal-spatial distribution dynamics of Sbp in *S. epidermidis* biofilms.

**Methods:** Biofilm formation and Sbp localization in *S. epidermidis* 1457 were investigated using (Live-)CLSM, STED microscopy, and MINFLUX nanoscopy to resolve spatio-temporal protein distribution upon surface attachment. Surface topography and matrix architecture were analyzed by AFM. Phenotypic characterization of 1457 and the corresponding sbp knock-out mutant (1457sbp) included growth curve analysis, biofilm adherence assays and comparative transcriptomic as well as proteomic profiling to assess stress response signatures. Extracellular reactive oxygen species (ROS) levels were quantified using colorimetric (NBT) assays. To identify Sbp interaction partners, proximity labeling and affinity-based ALFA-pulldown approaches were performed, followed by protein identification using proteomic analysis.

**Results:** Microscopy revealed that Sbp is produced immediately upon surface attachment of *S. epidermidis* 1457. Remarkably, Sbp spreads two-dimensionally across abiotic surfaces prior to bacterial colonization and is later incorporated into the intercellular matrix during three-dimensional biofilm development. AFM demonstrated that Sbp is essential for matrix architecture, indicating a Sbp-driven biofilm assembly process. Consistently, proximity-labelling and pull-down approaches identified multiple Sbp interaction partners (SIPs), supporting the concept of a superimposed biofilm matrix interactome. Enrichment of oxidoreductases among SIPs suggests a role in redox regulation, supported by increased ROS accumulation and growth defects in the *sbp* knockout mutant. Importantly, the growth defect of 1457 $\Delta$ *sbp* could be rescued by antioxidant treatment, demonstrating that elevated ROS levels underlie the observed phenotype.

**Conclusions:** This study provides dynamic insights into biofilm matrix assembly in *S. epidermidis* at single molecule resolution. Control of ROS detoxification by biofilm matrix components, recruiting moonlighting cytosolic proteins to the biofilm matrix, puts a novel and so far undiscovered piece to the puzzle of how biofilms ensure bacterial homeostasis to support chronic biomaterial associated infections.



## OP2.3 - EXPLORING TYPE IV PILI FUNCTION WITHIN BIOFILMS USING GENETIC CODE EXPANSION IN *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER BAUMANNII*

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**Background & Aims:** *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are opportunistic pathogens belonging to the ESKAPE group and are major causes of nosocomial infections worldwide. Their persistence in hospital environments and their ability to colonize host tissues are partly mediated by type IV pili (T4P), dynamic filamentous appendages involved in adhesion, twitching motility, biofilm formation, DNA uptake, and virulence. Despite their importance, the distribution and functional dynamics of T4P remain poorly characterized, particularly in *A. baumannii*, due to the lack of specific, non-perturbing labeling tools compatible with live-cell imaging. The aim of this work is to better understand the role of T4P in virulence and biofilm-associated dissemination by developing a bioorthogonal fluorescent labeling strategy. We hypothesize that T4P expression and activity are enhanced during the biofilm dispersion phase, a critical step in bacterial spread and colonization of new environments.

**Methods:** To specifically label T4P, we targeted PilA, the major structural subunit of the pilus. Genetic code expansion was implemented to incorporate an azide-containing non-canonical amino acid into PilA. The modified protein was subsequently labeled through strain-promoted azide-alkyne cycloaddition (SPAAC), enabling covalent and specific fluorescent tagging of T4P. Labeled bacteria were analyzed by fluorescence microscopy under planktonic and biofilm conditions.

**Results:** This strategy enables specific and reproducible fluorescent labeling of T4P in both bacterial species without affecting bacterial growth or pilus-associated functions. Observations suggest a differential distribution of T4P within the biofilm, supporting their regulated involvement in biofilm-associated processes.

**Conclusions:** This study introduces a bioorthogonal labeling approach compatible with live-cell imaging for the visualization of T4P in *A. baumannii* and *P. aeruginosa*. This tool opens new perspectives for investigating T4P expression, spatial organization, and dynamics during biofilm development and dispersion, contributing to a better understanding of T4P-mediated virulence mechanisms and their potential as therapeutic targets.



## OP2.4 - SURFACE-PROXIMITY-DEPENDENT REPROGRAMMING OF BIOFILM ARCHITECTURE INDEPENDENT OF ANTIMICROBIAL ACTIVITY

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1 - CytaCoat AB

**Background & Aims:** Biofilm development is initiated by surface sensing, which governs the transition from reversible microbial attachment to matrix-producing, three-dimensional community architecture. While most current strategies focus on microbial killing, surface-mediated regulation of phenotypic commitment remains poorly understood. We investigated whether a permanently bonded, superhydrophilic polymer-grafted surface modulates biofilm architectural maturation at a wound-material interface via altered surface sensing, and whether this effect extends to neighbouring ungrafted regions in a spatially confined, non-biocidal manner.

**Methods:** Biofilm formation by *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* was evaluated under simulated wound fluid (SWF) to replicate host-protein conditioning. Initial adhesion was quantified by colony-forming unit enumeration. Biomass was assessed using crystal violet staining. EPS architecture was visualised using Concanavalin A and EBBA Biolight to detect polysaccharide- and amyloid-rich matrix components. Three-dimensional biofilm organisation was analysed by confocal laser scanning microscopy (CLSM). Spatial effects were assessed by positioning grafted and ungrafted substrates in controlled proximity. Antimicrobial activity, extractables, leachables, and endotoxin release were evaluated using standardised assays.

**Results:** Initial adhesion and viable cell counts were comparable across substrates, confirming absence of antimicrobial activity. In contrast, grafted surfaces markedly altered biofilm maturation. Ungrafted materials supported dense, vertically organised, matrix-rich biofilms characterised by extensive intercellular bundling, hyphal integration (*C. albicans*), and amyloid-associated fibre formation (*P. aeruginosa*). Grafted regions exhibited attenuated microcolony formation, reduced multicellular aggregation, and failure to establish mature three-dimensional architecture despite preserved viability. Ungrafted substrates positioned in close proximity to grafted regions displayed graded reductions in matrix organisation and architectural complexity. This spatially confined effect was abolished beyond a defined separation distance, indicating short-range propagation of a surface-induced phenotypic shift rather than leaching or bactericidal action.

**Conclusions:** Interfacial cues can reprogram biofilm architectural commitment in a surface-proximity-dependent manner independent of microbial killing or adhesion inhibition. The conserved response across Gram-positive, Gram-negative, and fungal species supports a broadly acting surface-sensing mechanism capable of modulating community organisation beyond the directly modified interface. Surface-driven regulation of phenotypic commitment may represent an alternative paradigm for biofilm management based on modulation rather than eradication.



### OP3.1 - A PHYSIOLOGICALLY RELEVANT IN VITRO MODEL FOR STAPHYLOCOCCUS AUREUS BIOFILMS IN PROSTHETIC JOINT INFECTION

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**Background & Aims:** Prosthetic joint infections (PJIs) represent a major clinical challenge due to their chronicity and high rate of treatment failure, largely driven by the ability of *Staphylococcus aureus* to form biofilms. These structured bacterial communities provide protection from both host immune defenses and antibiotic therapies, thereby complicating diagnosis and treatment decisions. However, currently available *in vitro* biofilm models do not accurately reproduce the physiological conditions of the PJI environment, limiting their translational relevance.

**Methods:** To overcome this problematic, we developed an *in vitro* biofilm model integrating key parameters of the prosthetic joint niche. Biofilms were grown on suspended titanium pegs mimicking implant surfaces to prevent sedimentation of planktonic aggregates. Cultures were incubated under hypoxic conditions (2.6% O<sub>2</sub>) and in a bone-like environment medium (BLE+). A standardized protocol was established: bacterial adhesion for 8 h, followed by medium renewal to eliminate planktonic bacteria, and further incubation up to 72 h. Biofilm formation was assessed by viable cell counts, crystal violet staining, confocal laser scanning microscopy, and scanning electron microscopy.

**Results:** Kinetic analysis revealed the formation of mature biofilms at 72 h, with a decline in adherent bacteria thereafter. Hypoxia significantly increased bacterial adhesion compared to anoxia (0% O<sub>2</sub>), highlighting the importance of staying as close as possible to physiological oxygen concentrations. BLE+ medium supported robust biofilm growth. The suspended titanium pin system allowed the formation of structured biofilms, independent of aggregates sedimentation. Medium renewal after 8 h did not impair biofilm development, suggesting that biofilm growth was autonomous and not driven by planktonic bacteria. On this final model, eight clinical *S. aureus* strains (four MSSA and four MRSA) were then evaluated. Despite identical experimental conditions, pronounced polymorphism was observed, including differences in biofilm architecture, microcolony formation, biomass density, and proportions of viable bacteria.

**Conclusions:** This work allowed to establish an innovative and physiologically relevant *in vitro* model for studying *S. aureus* biofilms in the context of PJIs. By incorporating titanium surfaces, hypoxia, a bone-like medium, and a suspension system, this model allows fine phenotypic discrimination of biofilms and provides a robust platform for investigating persistence mechanisms and evaluating targeted anti-biofilm therapies.



## OP3.2 - NOVEL TECHNIQUES FOR STUDYING LEGIONELLA PNEUMOPHILIA ATTACHMENT

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1 - PHF Science

**Background & Aims:** *Legionella pneumophila*, the causative agent of legionnaires' disease, is a potentially life-threatening respiratory pathogen associated with engineered water systems such as plumbing, cooling towers, air conditioning systems, spas, decorative fountains and showerheads. The majority of sporadic and community-acquired cases of legionellosis cases worldwide are caused by *L. pneumophila* serogroup 1, though *L. longbeachae* can be associated with legionellosis outbreaks, particularly in Australia and New Zealand. Despite the global occurrence of legionellosis, many fundamental questions regarding *L. pneumophila* interactions with surfaces and biofilms in water systems remain unanswered or poorly understood.

**Methods:** In this work, we address two key questions regarding *L. pneumophila* attachment to surfaces, each using novel techniques. Firstly, we address the role of growth-dependent variations in *L. pneumophila* cell surface hydrophobicity and charge on adhesion to engineered surfaces using quartz-crystal microbalance with dissipation monitoring (QCM-D). Secondly, we address the lack of suitable surrogates for *L. pneumophila* by development of novel biopolymer surrogates and testing their adhesion to *Pseudomonas* biofilms.

**Results:** Cell surfaces of stationary phase *L. pneumophila* were significantly more hydrophobic than their lag and mid- exponential counterparts. QCM-D revealed lag and exponential phase *L. pneumophila* weakly adhered to stainless steel surfaces resulting in viscoelastic layers. In contrast, stationary phase bacteria were tightly and irreversibly bound to the surfaces, forming rigid layers. We also produced surrogates that mimicked *L. pneumophila*'s size, rod shape, surface charge, and hydrophobicity. Proof-of-concept validations of the surrogates were conducted in laboratory flow-through biofilm bioreactor experiments. The surrogate and *L. pneumophila* displayed similar attachment/detachment magnitudes and kinetics to/from *P. fluorescens* biofilms grown on stainless steel piping material. Their relative concentrations and peak attachment values were within the same orders of magnitude, and both exhibited slower attachment than detachment processes.

**Conclusions:** Legionnaire's disease is a global issue that requires new approaches, such as QCM-D and novel surrogates, to better understand *Legionella* attachment to surfaces and biofilms. Increased understanding of these processes will lead to better design of water treatment systems and development of new interventions to prevent growth and aerosolization of the organism, thereby preventing disease in the community.



### OP3.3 - USING THE GALLERIA MELLONELLA MODEL TO STUDY BURN WOUND BIOFILM FORMATION.

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**Background & Aims:** Burn wounds are a particularly complex trauma that are highly vulnerable to infection and there is a growing appreciation that there is a need for *in vivo* models to accurately recreate infection progression dynamics. It is particularly challenging, however, to obtain ethical approval for burn wound studies in mammalian models due to the high levels of suffering involved in the procedure. Previously, we developed an invertebrate model of burn wound trauma that recapitulates many of the hallmarks of burn wound trauma in humans. In this work we aim to develop the model further using *in vivo* transcriptomics and cross-scale multi-modal imaging to better understand biofilm formation across the entire burn wound site with unprecedented spatial detail.

**Methods:** Pathogenicity and wound dissemination studies are used to study the transition from biofilm associated to systemic infection. *In vivo* transcriptomics across multiple infection types is used to understand the specific transcriptional signatures associated with burn wound biofilm formation. Cross-scale multi-modal imaging of infected wound sites is used to quantify biofilm formation across the wound site.

**Results:** We initially confirm that bacteria colonise the wound site, forming biofilms before ultimately disseminating deeper into the host tissue and subsequently entering the *G. mellonella* open circulatory system. Through comparing the transcriptional signature in a wound infection to that observed in a systemic infection we identify key genes that are linked to biofilm formation specifically upregulated in the wound infection transcriptomic data. We subsequently use cross-scale multi-modal imaging to reveal the distribution of pathogens across the entire wound site, generating unprecedented 3D visualisations of the infection landscape; showing where pathogens colonise the wound, how far they penetrate into the wound bed, and the morphology of wound-associated biofilms in different species.

**Conclusions:** We demonstrate that the *Galleria* burn wound model has the capacity to recapitulate burn wound biofilms observed in higher mammalian models, with conserved transcriptional signatures. We outline a set of state-of-the-art approaches that can now be used to study pathogenicity in the burn wound microenvironment but also showcase a model that can be used at a high throughput capacity to screen for novel antibiofilm or antimicrobial therapeutics.



### OP3.4 - NON-INTERVENTIONAL REAL-TIME MONITORING OF BIOFILM FORMATION PROVIDES NEW INSIGHTS IN EXTRACELLULAR MATRIX GROWTH AND MATURATION

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<sup>1</sup> - Karolinska Institutet

**Background & Aims:** The extracellular matrix (ECM) is a hallmark of bacterial biofilms. The intricate structure and composition of the ECM protect from environmental stresses, including antibiotics and immune defences. Thus, the ability of bacteria to form biofilm affects clinical outcomes, but techniques to study the ECM are limited. Here, we develop novel methods for investigating the ECM to reveal insights into this critical aspect of the bacterial biofilm.

**Methods:** We present an agar-based method visualising ECM formation using the optotracer EbbaBiolight 680 alongside evaluation of colony growth dynamics in biofilms formed by Gram-positive (*S. aureus*) and -negative (*S. enteritidis*, uropathogenic *E. coli* (UPEC)) strains. Drop inoculation is used for macrocolony formation, spread-plating for single-cell derived colonies. Kinetic fluorescence spectroscopy combined with time-lapse microscopy reveal the spatial-temporal appearance of the ECM, confocal microscopy and 2-photon microscopy provides detailed characterization of biofilm microstructures. The wettability of biofilms is analysed by contact angle measurement, molecular analyses are performed, such as qPCR.

**Results:** Using automated fluorescence microscopy, we tracked the dynamics of biofilm growth in Gram-negative and Gram-positive species, showing the kinetics of bacterial growth (GFP) versus ECM-curli formation (EbbaBiolight 680). Through microscopy we identified the nano-architecture of UPEC and *S. aureus* biofilms where the matrix forms a protective layer over the bacterial cells. In UPEC biofilms it is highly hydrophobic, providing GoreTex-like protection to the cells and in *S. aureus* the cap-like structure produced in the biofilm is primarily composed of phenol-soluble modulins. Further, UPEC biofilms have distinct transcriptional zones showing high activity of curli and cellulose production. By up-grading the ColTapp software to include fluorescence analytics, we described in detail the formation of single cell derived biofilms in *S. aureus*. Collectively, our results reveal insights into the microarchitecture of biofilms, dynamics of their growth and transcriptional activity as well as development of software designed specifically for biofilm analytics.

**Conclusions:** Rapid, non-biased quantification of bacterial growth and ECM formation provides new means to streamline basic research into biofilm development and can contribute to the classification of clinical isolates. This approach can facilitate direct analysis of phenotypic heterogeneity within populations recovered from infected materials and support treatment decisions.



## OP4.1 - MOLECULAR RULES OF ENGAGEMENT: HOW SELF-ASSOCIATING AUTOTRANSPORTERS SHAPE BIOFILM ARCHITECTURE

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**Background & Aims:** Surface adhesins govern the earliest stages of biofilm development by mediating cell-cell and cell-surface interactions. Self-associating autotransporters (SAATs) are outer membrane proteins in Gram-negative bacteria that promote intercellular adhesion and aggregation, facilitating biofilm initiation. Despite their recognised role in community formation, the structural principles that control SAAT self-recognition and the extent to which interface variation shapes aggregation behaviour remain incompletely defined. We aimed to elucidate the molecular determinants of SAAT-mediated adhesion and define how these interactions influence early biofilm organisation.

**Methods:** High-resolution X-ray crystallography was used to resolve the passenger domains of representative SAATs, including Antigen 43 (Ag43) and TibA. Biophysical analyses characterised self-association in solution. Structure-guided mutagenesis identified key residues contributing to trans-association. Aggregation assays and quantitative biofilm assays under static and flow conditions were used to correlate structural determinants with adhesion strength and biofilm initiation. The effect of glycosylation on SAAT function was examined using genetic and phenotypic approaches. Structural insights were further used to develop molecular approaches specifically designed to target interface regions and disrupt SAAT-mediated biofilms.

**Results:** Structural analyses revealed a conserved head-to-tail interaction interface mediating specific trans-association between neighbouring cells<sup>1-5</sup>. Targeted mutation of interface residues significantly reduced aggregation and early biofilm biomass, confirming the functional importance of these contacts. Variation within interface regions modulated interaction strength, influencing aggregation kinetics and cluster compactness. Glycosylation of TibA reduced self-association efficiency, identifying a regulatory mechanism that fine-tunes intercellular adhesion at the surface. Targeting structurally defined interface contacts significantly reduced aggregation and biofilm formation, experimentally validating the functional centrality of these adhesion interfaces<sup>6</sup>.

**Conclusions:** Our findings define the molecular rules governing SAAT-mediated adhesion and establish a direct link between protein interaction energetics and biofilm architecture. Importantly, structure-guided targeting of these defined adhesion interfaces demonstrates that controlled perturbation of molecular contacts can modulate biofilm formation, providing a mechanistically grounded and experimentally validated framework for rational biofilm control

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## OP4.2 - DNA FROM GENOME TO CRITICAL EXTRACELLULAR VISCOELASTIC BIOFILM MATRIX COMPONENT THROUGH HIGHER ORDER STRUCTURAL CHANGES

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**Background & Aims:** While extracellular DNA (eDNA) is critical to biofilm formation, it is unclear why eDNA behaves differently to genomic DNA. In the extracellular matrix, DNA exists as fibers forming the foundational viscoelastic cross-linked structure of biofilms, and inside cells DNA is supercoiled and packaged, despite no differences in sequences. Understanding what contribute to the ability of eDNA to assemble into viscoelastic fibers is key to resolving processes contributing to eDNA release and biofilm formation. The aim here was to resolve higher order structures underpinning the eDNA matrix, using *Pseudomonas aeruginosa* as a model.

### Methods:

- Solid and solution state NMR on isolated, isotope labelled extracellular nucleic acids (NA).
- Immunoprecipitation of NA structures and sequencing (i.e. DRIP sequencing).
- Rheology with targeted structure-specific digestions.
- Corroborative immunofluorescence microscopy.

**Results:** We described firstly G-quadruplex eDNA and extracellular RNA (eRNA) as structural features of *P. aeruginosa* biofilms. G-quadruplex eDNA coincided with viscoelastic behaviors of biofilm and extracellular matrix, biofilm dissolution resulted from eRNA transesterification, and specific RNA appeared along eDNA fibers in the biofilm. Furthermore, pre-treating biofilms with low concentrations of DNase sensitized the biofilms to RNase digestion, suggesting that eRNA is bound to eDNA (i.e. e-DNA:RNA hybrid). R-loops are three-stranded nucleic acid structures comprising a DNA:RNA hybrid and single stranded RNA stabilized by G-quadruplexes. Extracellular (e-) R-loops were observed throughout *P. aeruginosa* biofilm extracellular matrices, including at key matrix structural nodes. Biofilm dissolution occurred only following hydrolysis of both DNA:RNA hybrids and single stranded DNA. e-R-loops were also observed in *Staphylococcus* biofilms and the lung infection patient sputum, highlighting clinical relevance. e-R-loops had a different gene profile to the transcriptome, and were thus in trans R-loops (i.e. forming away from transcription site). We observed stress response protein RecA in the nucleic acids matrix fibers and determined that recA is regulated by stringent stress response gene relA to insert toxic R-loops into the genome of a sub-population of suicide cells.

**Conclusions:** By employing a multidisciplinary approach, we thus described a mechanism for viscoelastic eDNA release resulting from activation of specific stress responses, whereby the R-loops are how cells die but also how they assemble their extracellular matrix.



### OP4.3 - POLYSACCHARIDE COMPONENTS PROVIDE THE MAJOR DIFFUSION BARRIER FOR NANO-SIZED OBJECTS IN GLYCAN-BASED BIOFILMS

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**Background & Aims:** Large, biopolymeric macromolecules have unique material properties, making them effective substances for embedding and shielding small objects from the environment. Bacteria use this strategy in biofilms where they secrete DNA, proteins, and glycans, in proportions that individually depend on their environment and life style. Plant pathogens such as *Erwinia* spp. must persist under water flow in the plant xylem and therefore produce anionic polysaccharides as the main biofilm component. This glycan-based material very efficiently binds water and forms a viscous network to slow down diffusion and transport of nano-sized objects. We present a single particle tracking approach to characterize glycan-based biofilms.

**Methods:** We have developed a fluorescence microscopy protocol to describe matrix viscosity via diffusion analyses of nano-sized beads to quantify polysaccharide entanglement, using a set of *Erwinia amylovora* and *Pantoea stewartii* strains with different biofilm polysaccharide chain lengths. Biofilms represent a major barrier against viral predation of bacterial hosts. We therefore investigated bacteriophage diffusion in the glycan biofilm matrices and found particle populations with distinct diffusion behavior.

**Results:** Our results show that phage diffusion is strongly influenced by biofilm concentration, pH, ionic strength, and tracer properties. Bacteriophages express glycan depolymerases as parts of their tails that rapidly degrade the polysaccharide matrix and we describe how these enzymes modulate particle dynamics within the matrix.

**Conclusions:** Our approach allows to rapidly assess biofilm polymer status and viscosity in microscopic set-ups for studies of biofilm progression, differentiation and dispersion. Moreover, we link analyzes of phage diffusion in viscous matrices to the dynamics of the phage-host infection process, which is key to get a more detailed understanding of bacteriophage-host coexistence in biofilms and an important starting point for using phage parts in anti-biofilm applications.



## OP4.4 - SPATIOTEMPORAL STAPHYLOCOCCUS AUREUS BIOFILM DYNAMICS IN A 3D IN VITRO PLATFORM

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**Background & Aims:** Conventional in vitro biofilm models rely on abiotic surfaces and nutrient-rich media, reinforcing a surface-centric paradigm that poorly captures the clinical reality of chronic, tissue-embedded infections. To overcome this limitation, we developed a modular three-dimensional (3D) platform based on gelatin methacryloyl (GelMA) hydrogels that recapitulate key mechanical and biochemical properties of host tissue microenvironments.

**Methods:** Single *Staphylococcus aureus* cells were encapsulated in soft tissue-mimetic GelMA (~3–5 kPa) at  $1 \times 10^5$  CFU/mL and cultured for 21 days in either Tryptic Soy Broth (TSB), a nutrient-rich standard biofilm medium, or Minimum Essential Medium supplemented with 10% serum (MEM), representing a defined and physiologically constrained nutrient environment. We integrated spatiotemporal confocal imaging, histology, flow cytometry, mechanical characterization (rheology and compression testing), and untargeted metabolomics to assess viability, biofilm architecture, matrix remodeling, and metabolic adaptation. To model the implant-tissue interface, 3D-printed polycaprolactone scaffolds were incorporated into the hydrogel matrix.

**Results:** The platform recapitulated the biofilm lifecycle—aggregation, maturation, and dispersal—while revealing pronounced nutrient-dependent phenotypes. Under TSB conditions, biofilms exhibited a hyperproliferative state characterized by rapid biomass expansion, large heterogeneous clusters, and extensive microenvironment remodeling, including protease-mediated hydrogel degradation and biomass-driven water redistribution. In contrast, MEM promoted a persistent phenotype consisting of smaller, compact, and mechanically stable aggregates. Metabolomic profiling revealed a fundamental metabolic shift: TSB-grown biofilms functioned primarily as extracellular nutrient scavengers, whereas MEM-grown communities exhibited biosynthetic autonomy, characterized by enhanced de novo nucleotide synthesis, upregulation of urea cycle and polyamine pathways, and accumulation of 2-hydroxyglutarate consistent with stress adaptation. Notably, incorporation of an implant surface did not override tissue-imposed constraints; although *S. aureus* colonized the scaffold locally, it did not transition to rapid surface-dominated growth typical of conventional assays.

**Conclusions:** Physiologically relevant 3D microenvironments fundamentally shape *S. aureus* biofilm architecture, mechanics, and metabolism. This platform captures spatial heterogeneity, extracellular matrix remodeling, and adaptive metabolic reprogramming that remain inaccessible in 2D systems, providing a robust framework to investigate chronic infection mechanisms and guide therapeutic development.



## OP6.1 - METABOLIC EVASEDROPPING: AMOEBA-DERIVED ACETALDEHYDE DRIVES BACTERIAL FLIGHT FROM AMOEBA PREDATION

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1 - Reichman University; 2 - Technion

**Background & Aims:** *Entamoeba histolytica* (Eh) is a major global intestinal pathogen responsible for significant mortality, yet the ecological dynamics governing its invasion of the gut microbiome remain poorly understood. Upon colonisation, Eh encounters resident bacteria predominantly organised as biofilms. While we have already established that *E. histolytica* utilises cysteine proteinases to degrade biofilm structural proteins, thereby accessing bacterial prey, however, the strategies bacteria employ to detect and evade this predation are largely uncharacterized.

**Methods:** We conducted an unbiased proteomic and metabolic analysis to determine how *B. subtilis* responds to amoeba lysate and secretions, with the ADH pathway either active or deactivated

**Results:** Our results indicate that *B. subtilis* detects this amoeba-derived metabolite, acetaldehyde, identifying it as a danger signal. Exposure to acetaldehyde produced by amoebae triggers a metabolic shift that inhibits the transcription of genes responsible for the bacterial extracellular matrix while enhancing chemotactic motility. As a result, acetaldehyde from amoebae prompts a rapid transition from a stationary biofilm state to a motile planktonic lifestyle.

**Conclusions:** These findings reveal that the bacteria exploit the Eh metabolic signature to coordinate a flight response. This interaction highlights how metabolic signalling shapes the gut microbiome population during parasitic amoeba infection.



## OP6.2 - DEVELOPMENT AND CHARACTERIZATION OF A MIXED PSEUDOMONAS AERUGINOSA-MYCOBACTERIUM ABSCESSUS BIOFILM MODEL FOR THE EVALUATION OF CLINICALLY RELEVANT ANTIBIOTICS AND NEW ANTIBIOFILM AGENTS FOR THE TREATMENT OF CF-RELATED CO-INFECTIONS

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**Background & Aims:** *Mycobacterium abscessus* (Mabs) is an emerging pathogen causing severe infections in cystic fibrosis (CF) patients, alongside the well-established threat of *Pseudomonas aeruginosa* (Pa). Both organisms form highly drug-tolerant biofilms in the CF lungs, contributing to treatment failure and chronic persistence, highlighting the urgent need for innovative strategies to limit their persistence and improve therapeutic outcomes. While these pathogens frequently cause CF-related co-infections, how interspecies interactions influence biofilm-associated antibiotic tolerance remains poorly understood. Addressing this question requires a robust *in vitro* model enabling the characterization of mixed-species biofilms and comparison of bacterial responses to antibiotics when grown alone or together. In this study, we developed an *in vitro* Mabs-Pa mixed biofilm model by identifying conditions that support stable co-growth as adherent biofilm cells. We characterized the model by populations quantifications and confocal laser scanning microscopy (CLSM) analysis. We next assessed the antibiotic susceptibility of two antibiotics and studied the effect of interspecies interactions on treatment responses.

**Methods:** We aimed to establish an *in vitro* mixed biofilm model by identifying conditions supporting the co-growth of both species as adherent biofilm while limiting planktonic proliferation. Planktonic growth was assessed in several synthetic media, including the Synthetic Cystic Fibrosis Medium (SCFM) that mimicks the CF lung conditions. Culture conditions were optimized to promote adhesion and the growth of single- and mixed- species biofilms. The biofilm populations were quantified by CFU enumeration and the biofilm architecture was visualized using CLSM. Finally, the antibiotic susceptibility to tobramycin and amikacin was assessed in mono- and co-cultures, on both planktonic and adhered cells.

**Results:** Optimized conditions enabled the adhesion and biofilm formation of Mabs in both Modified Biofilm Broth (MBB) and SCFM. CLSM imaging revealed distinct biofilm architectures depending on the growth medium. These conditions also supported the formation of mixed Mabs-Pa biofilms. Antibiotic assays showed increased tolerance of biofilm-associated cells compared with planktonic ones. Notably, mixed-species biofilms displayed distinct antibiotic tolerance profiles compared with monospecies biofilms when exposed to inhibitory and subinhibitory concentrations.

**Conclusions:** This optimized model provides a robust platform for high-throughput screening of anti-biofilm compounds combined with first-line antibiotics, paving the way for new therapeutic strategies against co-infections in CF.



### OP6.3 - INTRACELLULAR SURVIVAL OF PSEUDOMONAS AERUGINOSA AND STAPHYLOCOCCUS AUREUS IN A PHYSIOLOGICALLY RELEVANT CYSTIC FIBROSIS IN VITRO MODEL: IMPLICATIONS FOR ANTIBIOTIC INVASION AND INFECTION RELAPSE

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**Background & Aims:** Cystic fibrosis (CF) is a genetic disease characterized by the accumulation of thick mucus in the lungs, predisposing patients to chronic respiratory infections caused primarily by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Although classically considered extracellular pathogens, both bacteria possess the ability to internalize and survive within host airway epithelial cells. This intracellular niche acts as a reservoir, shielding the bacteria from immune clearance and antimicrobial therapies, which frequently leads to persistent and recurrent infections. This work aims to investigate the intracellular survival dynamics of these two pathogens using a physiologically relevant *in vitro* model closely mimic the CF airway environment, to gain insights into host-pathogens interplay and how it drives infection persistence and relapse in the respiratory tract.

**Methods:** An *in vitro* model recapitulating physiological key aspects of CF environment, combining A549 human pulmonary epithelial cells and artificial sputum medium, was infected with CF clinical isolates of *P. aeruginosa* and *S. aureus* (forming single and dual-species consortia) for 6 h. Following a gentamicin protection assay (1 h), cells were incubated for an additional 18 h in the absence and presence of antibiotics (ciprofloxacin and vancomycin), and subsequently, bacterial cells were quantified. Host response and host cell morphology, viability, and intracellular bacteria presence were evaluated via IL-8 levels and fluorescence microscopy (DAPI/WGA-FITC/PI).

**Results:** Following extracellular clearance, most strains successfully proliferated intracellularly and reappeared in the milieu mimicking infection relapse. During this process, CF isolates largely maintained their single-biofilm behaviour when integrated into polymicrobial consortia, with *P. aeruginosa* emerging as the dominant species. Furthermore, treatments with both antibiotics at minimal inhibitory concentrations failed to inhibit these intracellular pools, highlighting the protective nature of this niche. The host response was characterized by a robust, yet strain-dependent, pro-inflammatory IL-8 production, suggesting a differential capacity among strains to drive the damaging neutrophil-mediated inflammation characteristic of CF.

**Conclusions:** This study highlights that airway epithelial cells act as a protective reservoir for CF pathogens. The failure of standard antibiotics to eradicate internalized bacteria, driving subsequent cellular egress and relapse, underscores the urgent need for therapies specifically designed to target the intracellular microenvironment in CF.



## OP6.4 - REDUCED EFFICACY OF EFFLUX PUMP INHIBITORS AS ADJUVANT THERAPY IN POLYMICROBIAL BIOFILMS

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**Background & Aims:** Current in vitro biofilm models assessing the effectiveness of novel antimicrobials do not consider the impact of cohabiting microbes and environmental microniches often resulting in treatment failure. This leads to recurrent and chronic infections in patients with cystic fibrosis (CF). Understanding variations in antimicrobial tolerance and resistance mechanisms between mono-species and polymicrobial biofilms could go some way to avoiding this pitfall, thereby underpinning the development of novel therapeutic interventions in the future. We have previously developed accessible and highly adaptable polymicrobial biofilm models which mimic CF lung conditions that can be used to assess the impact of polymicrobial communities on the effectiveness of antibiotics. Using these, we aim to identify how the contribution of the *P. aeruginosa* RND efflux pumps differentially effects tolerance/resistance in polymicrobial biofilms compared to mono-species biofilms and the possibility of utilising efflux pump inhibitors to overcome the enhanced tolerance/resistance seen in polymicrobial biofilms.

**Methods:** We assessed the role of RND efflux pumps on the antimicrobial tolerance of *P. aeruginosa* biofilms grown with *Staphylococcus aureus* and *Candida albicans*. This was conducted using knockout *P. aeruginosa* mutants of four multidrug efflux pumps associated with increased tolerance to antibiotics. These were tested for sensitivity to meropenem and tobramycin, commonly used to treat CF-related infections. Minimal biofilm inhibitory concentrations were determined as colony-forming units and metabolic activity.

**Results:** From these mutants, only the MexAB-OprM knockout mutant revealed significant effects on *P. aeruginosa* planktonic and biofilm tolerance to meropenem and tobramycin combination therapy. In contrast, in polymicrobial biofilms, the MexAB-OprM mutant did not show increased antimicrobial sensitivity to either mono-therapy or combination therapy. In agreement with these findings, MexAB-OprM specific and non-specific efflux pump inhibitors increased tolerance of *P. aeruginosa* to antibiotics in single species biofilms but were significantly less effective in a polymicrobial context.

**Conclusions:** This work indicates there is potential redundancy in efflux pump-mediated antibiotic tolerance due to the loss of sensitivity and effectiveness of efflux pump inhibitors possibly due to increases in alternate tolerance/resistance mechanisms that are only enhanced in polymicrobial biofilms. It also suggests that there are restrictions in using efflux pumps as adjuvant therapy in polymicrobial biofilms.



## OP7.1 - MICROPLASTIC PARTICLES AND PLASTIC ADDITIVES PROMOTE BIOFILM FORMATION, VIRULENCE AND ANTIBIOTIC RESISTANCE IN *VIBRIO CHOLERAE*

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**Background & Aims:** Plastic waste is ubiquitous in ecosystems and poses an increasing ecological challenge with unpredictable consequences. Microplastics (MP), i.e. particles smaller than 5 mm, serve as a surface for microorganisms to form biofilms, the so-called "plastisphere", which favors certain species and can thus disturb the ecological balance. The colonization of microplastics by pathogenic bacteria, e.g. the cholera-inducing *Vibrio cholerae*, poses a clinical threat as these bacteria-carrying MP particles can enter the food chain and biofilm-associated bacteria also display increased antibiotic resistance. The aim of this study was to elucidate the role of MP particles as vectors for pathogenic bacteria, such as *V. cholerae*, and to identify the underlying mechanisms driving increased antibiotic resistance in MP-associated biofilms.

**Methods:** To understand the mechanisms underlying biofilm formation of *V. cholerae* on MP, we employed confocal microscopy with automated image analysis, including a microfluidic chip model for live monitoring of biofilm formation and investigation of factors impacting biofilm integrity. We developed an intestine-on-chip model to systematically investigate the role of plastic particles as vectors for pathogenic bacteria, such as *V. cholerae*, and to assess their effects on the human host. Further, we used transcriptome analyses to understand the effects of plastic additives on the plastisphere at the molecular level.

**Results:** Our results show a robust formation of biofilms by *V. cholerae* on MP which enhances the attachment of MP to the epithelium in the intestine-on-chip. Considering that the continuous degradation of plastic debris in the environment causes leaching of chemical plastic additives such as bisphenol A (BPA), we further investigated the effects of BPA on the formation of these microplastic associated biofilms. Here, we observed that BPA triggers *V. cholerae* to form thicker and more enduring biofilms on MP. Applying transcriptome analysis, we identified a distinct response of *V. cholerae* to the presence of BPA, such as an increased transcription of RND-type efflux pumps as well as activation of stress-response and resistance pathways that enhance bacterial resistance to  $\beta$ -lactam antibiotics.

**Conclusions:** Our study suggests that the plastic additive BPA enhances biofilm persistence and antibiotic resistance in *V. cholerae*, thereby contributing to the negative effects of microplastic-associated biofilms.



## OP7.2 - PATHOGENIC BACTERIA ARE ELECTROGENIC AND USE CATALYTICALLY ACTIVE DNA STRUCTURES FOR EXTRA-CELLULAR ELECTRON TRANSFER AND PROTECTION AGAINST OXIDATIVE STRESS

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**Background & Aims:** Extracellular DNA is essential for biofilm formation in many bacteria. It contributes to the initial adhesion, and increases the elasticity of mature biofilms. In recent years, it has come to light that extracellular nucleic acids in biofilms comprise both DNA and RNA, and that non-canonical structures like G-quadruplexes (G4) and the left-handed double helix Z-DNA can be highly abundant. G4 provide emergent properties to biofilms as they bind hemin to form a catalytically active DNzyme with peroxidase-like activity. The aim of this research was to understand the biological significance of extracellular G4/hemin complexes in biofilms. We hypothesize that electrons can be transferred from bacterial metabolic processes to the ex- tracellular G4/hemin complex, enabling electrogenic properties and reduction of harmful reactive oxygen species at the cell surface.

**Methods:** We visualized G4 in *Staphylococcus epidermidis* and *Staphylococcus aureus* biofilms grown with/without amendment of G4 oligos using fluorescence immunolabeling and confocal laser scanning microscopy. The extracellular peroxidase activity of the G4/hemin complex was visualized by tyramide signal amplification. Electrogenic activity was quantified by depositing planktonic bacteria or growing biofilms directly on screen-printed electrodes and performing cyclic voltammetry (-0.8-0.8 V) and differential pulse voltammetry at 0.4 V. Intracellular reactive oxygen species in bacteria with/without G4/hemin was visualized by fluorescence labelling using CellROX™ after aerobic incubation in buffer for up to 24 h.

**Results:** *S. epidermidis* and *S. aureus* produced G4 in the biofilm and associated with the cell surface. *S. epidermidis* and *S. aureus* both displayed electrogenic activity, enabling electron transfer to/from an electrode poised at 0.4 V when G4/hemin was present. Increased amount of G4/hemin on the bacterial surface resulted in less reactive oxygen species in the cells when incubated aerobically in buffer, indicating that the DNzyme activity provides protection against oxidative stress.

**Conclusions:** Extracellular nucleic acids form G4 structures on the surface of staphylococci and in the extracellular matrix of biofilms. In the presence of hemin that is available in the infectious microenvironment, these structures form a catalytically active complex which acts as a conduit for electron transfer and as catalyst to protect bacteria from oxidative stress.



### OP7.3 - EFFLUX-MEDIATED CROSS-RESISTANCE AND COLLATERAL SENSITIVITY BETWEEN ANTIBIOTICS AND BIOCIDES DURING SURFACE-ASSOCIATED GROWTH OF *PSEUDOMONAS AERUGINOSA*

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**Background & Aims:** Wounds, clinical surfaces, as well as wastewater from hospitals and livestock, represent environments in which bacterial communities are exposed to selective concentrations of an antimicrobial cocktail, consisting of antibiotics, antiseptics and biocides. While it is known that antibiotic-antibiotic combinations can lead to evolutionary interactions, driving either cross-resistance or collateral sensitivity, such combination effects of antibiotics with biocides or antiseptics in the context of clinically relevant antibiotic resistance mutations in *Pseudomonas aeruginosa* MPAO1, involving the upregulation of efflux (*DnfxB*, *DmexZ*, *DmexR*) and the decrease of drug influx by deleting an outer membrane porin (*DoprD*).

**Methods:** We used a fluorescence-based agar screen and biofilm flow chamber experiments to examine how concentration gradients of the biocides octenidine (OCT), benzalkonium chloride (BAC), cetrimonium bromide (CTAB), povidone-iodine (PVP-I), chlorhexidine digluconate (CHX) and silver nitrate (AgNO<sub>3</sub>) interact with subinhibitory concentrations of antibiotics (meropenem, ciprofloxacin and gentamicin) to select for or against antibiotic-resistant mutants. In addition, knock-out mutants were constructed and further characterized by whole-genome re-sequencing, broth microdilution MIC assays including dose-response analysis and efflux inhibition.

**Results:** Our results show that combinations of antibiotics and biocides/antiseptics generate detectable selection outcomes in antibiotic-resistant mutants. Both porin deletion and efflux derepression generate mutation- specific combination effects, with efflux effects being biocide- and pump-specific. Specifically, we find that cross-resistance against PVP-I is linked to overexpression of MexXY-OprM (*DmexZ*), suggesting that PVP-I is a possible substrate of the MexXY-OprM efflux pump. In contrast, cross-resistance against chlorhexidine by derepression of the efflux pump MexCD-OprJ (*DnfxB*) appeared to be efflux independent. Biofilm flow chamber experiments with chlorhexidine and meropenem showed intricate spatial interactions of wildtype and efflux overexpressing, antibiotic-resistant cells. Lastly, we identified selective concentration windows of biocides, which can select for or against antibiotic resistance.

**Conclusions:** Our findings highlight that biocide-antibiotic combinations can shift selection dynamics in mutation-specific and efflux-dependent ways. Understanding these interactions is essential to reduce the risk of co-selection of antibiotic resistance upon use of biocides and antiseptics, but also to design rational use strategies of biocides and antiseptics to select against antibiotic resistance.



## OP7.4 - GLOBAL TRANSCRIPTOMIC PROFILING OF PSEUDOMONAS AERUGINOSA PAO1 DYNAMIC BIOFILM

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**Background & Aims:** *Pseudomonas aeruginosa* is known for its high adaptability to environmental changes, particularly through biofilm formation (Flemming *et al.*, 2010). This lifestyle is involved in resistance mechanisms and tolerance to various treatments like antibiotics for example (Williamson *et al.*, 2012; Walters *et al.*, 2003). It is essential to identify the transcriptional modulations promoting biofilm formation on both abiotic and biotic surfaces in clinical contexts. The aim of this study is to characterize the differential gene expression between planktonic cells and biofilm in dynamic conditions by RNA sequencing.

**Methods:** RNA sequencing allows the capture of the transcriptomic response at a given instant in time. *Pseudomonas aeruginosa* PAO1 cultures were grown in liquid culture for planktonic condition and in flow-cell chambers for the biofilm condition. This technic allows bacterial growth in a dynamic environment, mimicking conditions from environment. Differential gene expression analysis was performed using the Galaxy platform and various databases were used to identify the pathways associated with the differences between these two lifestyles.

**Results:** A total of 2625 genes were found to be differentially expressed when comparing transcriptomes of the two lifestyles, including 1283 downregulated and 1342 upregulated genes in biofilm. These differences highlight a global rearrangement of bacterial metabolism, confirming results of previous studies (Dietrich *et al.*, 2008; Stewart and Franklin, 2008), as well as the establishment of a specific regulatory network that recruits fewer extracytoplasmic sigma factors within a biofilm. The dynamic conditions of biofilm formation also appear to promote the establishment of virulence factors such as type III and VI secretion systems.

**Conclusions:** This study provides the first global transcriptomic profile of *Pseudomonas aeruginosa* PAO1 biofilms cultured in continuous-flow cells, capturing the real-time adaptive responses to fluid dynamics that define certain chronic infections. It identifies the major pathways and mechanisms involved in this lifestyle. These findings improve our understanding of *Pseudomonas aeruginosa* biofilm and help to identify potential targets for new strategies to disrupt biofilm formation or persistence.



## OP7.5 - A MULTIMODAL THERAPY INTEGRATING ULTRASOUND-STIMULATED MICROBUBBLES, ANTIBIOFILM AGENTS, AND ANTIBIOTICS AGAINST EARLY-STAGE AND MATURE BIOFILMS

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**Background & Aims:** Biofilm-associated infections (BAIs) account for up to 80% of human infections, often contributing to chronicity and antibiotic resistance. Biofilms, commonly found in chronic wounds, create a diffusional barrier that limits antimicrobial penetration and provide a niche for dormant and antibiotic-tolerant bacterial populations. Ultrasound (US)-stimulated microbubbles (MBs) have shown promise for biofilm disruption, yet their integration with chemical antibiofilm agents and antibiotics has not been systematically explored. This study reports, for the first time, the combined application of MBs with antibiofilm agents tetrasodium ethylenediaminetetraacetic acid (T-EDTA) or nitric oxide (NO)-releasing NONOates (PAPA NONOate; PA- NO and spermine NONOate; SP-NO), with the antibiotic ciprofloxacin against *Pseudomonas aeruginosa* PAO1 biofilms of varying maturity.

**Methods:** *P. aeruginosa* PAO1 biofilms were cultured in LB for either 24 or 48 h at 37 °C to model early-stage and mature biofilms, respectively. Perfluorobutane core, phospholipid MBs were exposed to varying acoustic pressures to determine the optimal ultrasound exposure conditions for biofilm removal. Samples were exposed to US for 40 s at 1.1 MHz with tone bursts of 400 cycles and 20 Hz pulse-repetition frequency reaching focal peak-negative pressures between 0.25-1 MPa. MBs were then co-administered with 250 µM NONOates, or 4% w/v T-EDTA either in the absence or presence of 0.25 µg/mL ciprofloxacin (i.e. inhibitory); samples were exposed to 1 MPa acoustic pressure. Biofilms were analysed by using LIVE/DEAD staining and fluorescence microscopy to determine total biofilm coverage area.

**Results:** Among all regimens, T-EDTA combined with ciprofloxacin and MBs achieved the most consistent and substantial biofilm removal (>70%), highlighting multimodal efficacy of combining physical disruption, matrix modification, and antibiotic action. NONOates showed limited benefit when paired with MBs, suggesting cavitation does not enhance NO-mediated dispersal, although the addition of ciprofloxacin did improve efficacy. Biofilm maturity (24 vs. 48 h) significantly influenced treatment outcomes, with early-stage biofilms responding more readily to cavitation-driven disruption. For all treatments, the addition of ciprofloxacin improved outcomes indicating a potentiation effect.

**Conclusions:** Findings from this study suggest that the combination of biofilm dispersal compounds with cavitation-mediated approaches represents a promising strategy for addressing the challenges of BAIs, particularly through enhancement of antimicrobial delivery and efficacy.



## OP7.6 - SPRAYABLE HYALURONIC ACID-POLYARGININE SUPRAMOLECULAR FILMS FOR RAPID, ANTIBIOTIC-FREE PREVENTION OF WOUND AND DEVICE BIOFILMS

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**Background & Aims:** Biofilm-associated infections in wounds and on medical devices are notoriously difficult to prevent and treat, particularly given the increasing challenge of antibiotic resistance. To address this, we developed a rapid, point-of-care, antibiotic-free coating strategy designed to conformally protect both living tissues and clinically relevant device materials by preventing early biofilm formation.

**Methods:** A portable dual-syringe spray system was engineered to co-deliver oppositely charged hyaluronic acid (HA) and polyarginine (PAR30), enabling *in situ* formation of a thin polyelectrolyte complex nanometric film on diverse surfaces without intermediate rinsing. Antimicrobial activity was evaluated *in vitro* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* using optical density and viable count assays, including agar-based models. Efficacy was further tested in a murine wound infection model using bioluminescent methicillin-resistant *S. aureus* (MRSA). Biocompatibility assessments followed ISO 10993-5 direct-contact cytotoxicity testing on Balb/3T3 cells, and *in vivo* inflammatory monitoring through interleukin-6 (IL-6) in murine wound models. Post-surgical outcomes, including pain-related behaviors and preservation of motor function, were evaluated in a mouse incision model. Environmental safety was assessed through tiered ecotoxicity assays.

**Results:** Spray deposited HA-PAR30 films adhered robustly to multiple substrates and demonstrated strong contact- active antimicrobial activity, achieving substantial reductions in bacterial burden both *in vitro* and *in vivo*. Film morphology and uniform coverage (~2–3 µm thickness) were confirmed via FIB-SEM/EDS on titanium and glass. In murine wounds, treated sites exhibited markedly reduced MRSA bioluminescence and lower bacterial loads compared with uncoated controls, reflecting prevention of early colonization (> 6-log reduction). The formulation met biocompatibility criteria, with cell viability above ISO thresholds, and no increased inflammatory responses relative to untreated wounds. Beyond infection control, HA/PAR30-coated incisions demonstrated improved post-surgical outcomes, including reduced pain and preservation of motor function in mice. Environmental safety was assessed using aquatic (*Tetrahymena thermophila*, *Daphnia magna*) and terrestrial (*Porcellio scaber*) organisms, showing minimal toxic effects.

**Conclusions:** Developed under a sustainable-by-design framework, this dual-syringe spray enables rapid *in situ* assembly of supramolecular antimicrobial films, offering a practical, antibiotic-free strategy for early biofilm control. It combines broad-spectrum efficacy, with biocompatibility, and practical delivery compatible with time-constrained clinical workflows.



## OP8.2 - EVALUATION OF GENE EXPRESSION MARKERS FOR DIAGNOSIS OF BACTERIAL VAGINOSIS: A GARDNERELLA-SPECIFIC TRANSCRIPTIONAL PROFILE

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**Background & Aims:** Bacterial vaginosis (BV) is the most common vaginal infection among women of reproductive age. It is characterized by a reduction of protective *Lactobacillus* species and an increase of anaerobic species that form a polymicrobial biofilm on the vaginal epithelium. *Gardnerella* species are the most prevalent species in cases of BV. However, transcriptomic alterations that occur during the development of BV remain poorly understood. This study aimed to evaluate the potential of *G. vaginalis* genes as molecular markers for BV diagnosis, by quantifying gene expression in vaginal specimens from women who developed incident BV (iBV) and healthy women.

**Methods:** Vaginal specimens were collected longitudinally from 18 women with baseline normal vaginal microbiota who provided twice daily self-collected vaginal specimens for 60 days or until iBV (Nugent score 7-10 on  $\geq 4$  consecutive specimens). BV cases were matched 1:1 to women maintaining optimal vaginal microbiota for the majority of the study (controls). RNA was extracted from six specimens for each BV case collected in the three days before the development of iBV, the day of iBV diagnosis, and the two days after; control specimens days selected for RNA extraction were matched by menses date. The expression of four *G. vaginalis* genes was quantified using qPCR. The microbial composition of each specimen was assessed using 16S rRNA sequencing targeting the V4 hypervariable region.

**Results:** The evaluation of genes' potential for BV diagnosis showed that sensitivity varied across targets: two genes were detected in  $< 20\%$  of iBV participants, while another two genes were detected in 40% and 60% of iBV participants, respectively. Notably, expression of three of the four genes was detected in only one sample within the 102 control specimens. The 16S rRNA sequencing results showed a clear difference in the composition of BV cases and controls; BV cases had high species diversity, while controls were predominantly dominated by *Lactobacillus* species.

**Conclusions:** The detection of three of the four targeted genes in only one of the healthy controls suggests the potential of these genes to be used as diagnostic markers to discriminate between BV-positive and BV-negative cases.



### OP8.3 - POLYMICROBIAL COMMUNITIES DRIVE BIOFILM FORMATION AND EXACERBATE HOST RESPONSES IN A 3D HUMAN BLADDER MICROTISSUE MODEL

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**Background & Aims:** Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide, accounting for hundreds of millions of cases annually and contributing substantially to rising antimicrobial resistance and UTI-associated mortality. Biofilm formation is a key driver of persistence and treatment failure in chronic and device-associated infections; however, its role in non-catheter-associated UTIs remains poorly defined. Emerging evidence suggests that biofilm-like communities may form within the bladder and promote recurrence and poor therapeutic outcomes. Although polymicrobial biofilms are common in chronic infections, their contribution to UTI pathogenesis has been largely overlooked, partly due to limitations in physiologically relevant experimental models.

**Methods:** To investigate polymicrobial interactions under host-relevant conditions, we evaluated the growth fitness and biofilm-forming capacity of clinically-relevant uropathogens cultured as mono-, dual-, and triple-species communities. Experiments were performed in pooled human urine and conventional laboratory media to assess environmental effects on microbial dynamics. Biofilm formation and host responses were further examined using a three-dimensional, urine-tolerant human urothelial microtissue model (3D-UHU). Host- pathogen interactions were assessed by quantifying urothelial exfoliation, cytotoxicity, epithelial barrier integrity, and cytokine production following mono- and polymicrobial infection. In parallel, untargeted metabolomics analysis was performed to compare metabolic profiles of mono- versus polymicrobial communities in the 3D-UHU.

**Results:** Microbial fitness and interspecies interactions were strongly influenced by environmental cues. We found that several uropathogens competed in nutrient-rich laboratory media, but coexisted in urine culture. Stable dual- and triple-species biofilms formed within the 3D-UHU model, displaying distinct spatial organization and community architecture compared with monospecies biofilms. Polymicrobial infection induced significantly enhanced host responses, including increased urothelial exfoliation, elevated cytotoxicity, impaired epithelial barrier function, and heightened inflammatory cytokine secretion. Untargeted metabolomic analyses revealed distinct metabolic signatures associated with polymicrobial communities, indicating altered metabolic activity compared with monospecies biofilms.

**Conclusions:** The 3D-UHU model enables physiologically relevant investigation of polymicrobial biofilms in UTIs, and reveals amplified host damage and metabolic reprogramming during multispecies infection. We also demonstrate that human urine is a critical determinant of uropathogen-uropathogen interactions and biofilm behaviour. This work underscores the importance of incorporating realistic host environments and microbial complexity to better understand UTI pathogenesis and treatment failure.



## OP8.4 - BEYOND SINGLE PATHOGENS: WHAT MIXED CO-INFECTIONS REVEAL ABOUT CHRONIC LUNG DISEASE

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**Background & Aims:** Chronic lung diseases like cystic fibrosis, COPD, and bronchiectasis are recognised as polymicrobial environments where pathogens, including *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, and *Mycobacterium abscessus*, form complex, multispecies biofilms. These mixed communities influence antimicrobial tolerance, regulate host immunity, and promote ongoing inflammation, which can worsen disease progression. The interactions between co-infecting species result in emergent behaviours not seen in single-species infections, posing new challenges for diagnosis and treatment.

**Methods:** We studied pathogen-pathogen and pathogen-host interactions through dual-species biofilm assays, bronchial epithelial cell infection models, and in vivo experiments in *Galleria mellonella*. Our analysis included infection progress, host immune response, bacterial virulence gene activity, and antibiotic resistance during co-infections with acute and chronic *P. aeruginosa* strains, combined with *M. abscessus* and *B. cenocepacia*.

**Results:** Coinfections consistently produced more severe outcomes than single-species infections. In biofilms, both mixed biofilm pairs formed stable mixed biofilms with reciprocal inhibition of their respective single-species biofilm growth. In epithelial cells, mixed infections significantly decreased cell viability compared to single-pathogen exposures. In vivo, using *G. mellonella*, coinfections reduced host survival and heightened disease symptoms. *B. cenocepacia* suppressed melanization and immune activation, while *P. aeruginosa* triggered strong pro-inflammatory responses. Conversely, *M. abscessus* dampened the immune response during co-infection, worsening host outcomes. In mixed infections, upregulated virulence genes were found in all species. Interestingly, co-infected groups showed increased antibiotic susceptibility, indicating unexpected vulnerabilities in polymicrobial interactions.

**Conclusions:** Mixed co-infections fundamentally reshape infection dynamics in chronic lung disease by modulating biofilm behaviour, virulence, and host immunity. The contrasting immune modulatory strategies of *P. aeruginosa*, *B. cenocepacia*, and *M. abscessus* produce synergistic pathogenic effects that cannot be predicted from single-species studies. These findings highlight the potential for exploiting interspecific interactions to improve therapeutic strategies for chronic lung infections.



## OP10.1 - RESPONSE OF RESISTANT CANDIDA ALBICANS BIOFILMS TO PDI-INDUCED OXIDATIVE STRESS

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**Background & Aims:** *Candida albicans* biofilm poses a significant clinical challenge due to its high resistance to conventional treatments. Photodynamic inactivation (PDI) has emerged as a promising strategy to overcome this resistance. In oxygen-rich conditions, photosensitizers (PS), such as methylene blue (MB) or phloxine B (PhB), generate reactive oxygen species (ROS) upon light irradiation. These ROS cause oxidative damage, leading to a marked reduction in biofilm integrity and cell viability. The question is whether PDI can overcome efflux-mediated resistance in *C. albicans*. Of particular interest is the regulation of ROS-detoxifying genes following PDI and whether the yeast-to-hyphae transition modulates the fungal stress response.

**Methods:** The standard *C. albicans* strain SC5314, a *cph1/efg1* double mutant defective in filamentation, a fluconazole-resistant *C. albicans* strain, and methicillin-resistant *Staphylococcus aureus* (MRSA) were used. PDI efficacy was tested on biofilms formed in 96-well microtiter plates (MB) or on poly(urethane) disks functionalized with layered silicate saponite and PhB. Biofilm growth was evaluated by colony-forming units (CFU/mL) and PMA-qPCR. Expression of efflux and ROS-detoxifying genes was analyzed by qPCR. ROS production and the GSH/GSSG ratio were measured using commercial assays. Biocompatibility of PSs was assessed using the *in vivo* model organism *Galleria mellonella*.

**Results:** PDI markedly reduced the viability of *C. albicans* biofilm cells, even in strain with increased expression of *CDR* and *MDR1* efflux genes, but efficacy depended on irradiation duration. A nanocomposite with PhB decreased the survival of mixed *C. albicans*-MRSA biofilms by approximately 100-fold. SEM confirmed structural damage, and H<sub>2</sub>O<sub>2</sub> measurements indicated a significant increase in ROS production after PDI. Treatment also altered the GSH/GSSG ratio, with the most pronounced changes observed in thioredoxin pathway genes involved in ROS detoxification. The SC5314 strain, capable of forming hyphae, displayed lower susceptibility to oxidative stress compared to the filamentation-defective mutant, whereas even the resistant strain was more susceptible to PDI, highlighting the superior capacity of the mycelial form to respond to PDI-induced oxidative stress.

**Conclusions:** PDI effectively reduces *C. albicans* biofilm viability and induces oxidative stress that overcomes efflux and detoxification mechanisms, whereas the mycelial form shows increased stress tolerance. This work was supported by the projects: APVV-21-0302, APVV-22-0150; VEGA 1/0240/23, VEGA 1/0484/24.



## OP10.2 - INVESTIGATING THE EFFECTS OF DISINFECTANTS AGAINST DRY AND HYDRATED CANDIDOZYMA AURIS BIOFILMS

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**Background & Aims:** *Candidozyma auris* is a problematic fungal pathogen on the WHO's Critical Priority group of fungal pathogens. Of key concern is its ability to cause outbreaks within intensive and chronic care units, which is facilitated through its environmental persistence. This current study aims to understand the role that biofilms play in surviving disinfection using a dry surface biofilm (DSB) model.

**Methods:** Representative *C. auris* clinical isolates obtained from a prospective surveillance in Scotland were grown in a hydrated and dehydrated biofilm model. Disinfection was performed, as per manufacturer's instructions, using clinically relevant protocols of commercial sodium hypochlorite (NaOCl) (Actichlor Plus; Ecolab, UK), peracetic acid (Clinell Enhanced Pods; GAMA Healthcare Ltd, UK) and chlorine dioxide (Tristel Fuse; Tristel Solutions Ltd, UK). Disinfectant efficacy against biofilm was evaluated using conventional cell counting. Post treatment glove transmission of *C. auris* biofilms was also evaluated.

**Results:** Hydrated biofilms (HBs) and dry surface biofilms (DSBs) exhibited equivalent numbers of recoverable cells. When treated with each disinfectant, HBs were significantly susceptible to treatment by up to 6 logs reduction, though Tristel Fuse was ineffective. Activity of disinfectants against DSBs were significantly diminished compared to the effects against HBs. DSBs were able to regrow with media replenishment, whereas in Actichlor Plus and Clinell Enhanced Pods treated HBs regrowth was suppressed. Touch transmission of DSBs was significantly more prominent compared to HBs.

**Conclusion:** We have optimised a DSB protocol in which we show persistence of *C. auris* after disinfectant treatments. The ability for these disinfected biofilms to regrow and be transmissible when rehydrated remains an important consideration for infection prevention and control guidelines. The equivalency of the neutral pH peracetic (Clinell Enhanced Pods) offers a more surface friendly option compared to harmful sodium hypochlorite solutions.



### OP10.3 - WITHIN-HOST ADAPTATION DURING RELAPSING INFECTIVE ENDOCARDITIS SELECTS FOR INCREASED BACTERIAL RESILIENCE

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**Background & Aims:** *In vivo* evolution provides pathogens with opportunities for within-host adaptation, which have been associated with poor infection control and treatment failure. However, understanding the mechanisms induced by mutations that arise during infection, and linking these changes to treatment outcomes, remains challenging. Here, we aimed to characterize the genetic and phenotypic changes associated with within-host adaptation in a case of relapsing infective endocarditis (IE) with *Enterococcus faecalis*. All *E. faecalis* isolates collected during the infection course were sensitive to ampicillin and gentamicin and the patient received guideline-recommended dual antibiotic regimens (ampicillin and gentamicin) for 4–6 weeks for IE. Despite this, the patient experienced multiple relapses with increasingly persistent bacteremias, ultimately necessitating valve replacement surgery to achieve treatment success.

**Methods:** Longitudinal bacterial isolates were collected from the four bacteremia episodes. These isolates were analyzed with whole-genome sequencing and phenotypic assays to evaluate key bacterial traits indicative of within-host adaptation, including antibiotic susceptibility, antibiotic tolerance, growth dynamics, and biofilm formation.

**Results:** Analyzing clones isolated from different episodes of bacteremia revealed the emergence of a small colony variant (SCV) in the 3rd bacteremia. The SCV carried a mutation in 6-phosphogluconate dehydrogenase (*gnd*) in the pentose phosphate pathway. Mutations affecting the central carbon metabolism have previously been associated with growth defects and increased antibiotic tolerance. Consistent with this, comparison of the SCV with isolates obtained earlier in the clinical course demonstrated markedly increased tolerance to ampicillin and gentamicin, a substantial growth defect (50% fitness decrease), and increased biofilm formation. Despite these phenotypic changes, the SCV exhibited unchanged antibiotic susceptibility profiles for ampicillin and gentamicin.

**Conclusions:** Here, we describe the *in vivo* evolution of *E. faecalis* during IE, where an acquired mutation led to a slow-growing, antibiotic-tolerant phenotype that may have contributed to multiple relapses. These findings highlight the importance of understanding within-host evolutionary dynamics during systemic infections to improve clinical management and outcomes.



## OP10.4 - CONTRIBUTION OF THE BIOFILM EXTRACELLULAR MATRIX TO SALMONELLA GENE REGULATION AND OXIDATIVE STRESS RESPONSES

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**Background & Aims:** Salmonella is a zoonotic pathogen with a cyclic lifestyle in which survival and adaptation to different environments is entirely dependent on its ability to form biofilms. In Salmonella enterica, the biofilm master regulator CsgD drives production of pEtN-cellulose and curli fimbriae, the main components of the biofilm extracellular matrix. Previous transcriptomic studies have associated CsgD with metabolic remodeling and stress responses within biofilms. Because CsgD expression coincides with matrix production, this study aimed to disentangle the specific contributions of CsgD and the biofilm matrix to global gene regulation during biofilm formation.

**Methods:** The transcriptional profiles of a wild type strain, a csgD mutant, and a biofilm matrix-deficient mutant were compared after incubation under biofilm inducing conditions. Differentially expressed genes (DEGs) regulated by CsgD and/or the matrix were identified. Selected genes were deleted, and resulting mutants were evaluated for survival, matrix synthesis, and resistance to external stresses during biofilm formation.

**Results:** RNA-seq analysis revealed a strong overlap between DEGs within the CsgD regulon and those differentially expressed in the absence of the matrix. Many shared DEGs were linked to oxidative stress responses. Consistently, intracellular ROS quantification demonstrated increased oxidative stress in biofilm-forming cells relative to mutants unable to produce the biofilm matrix. Uptake of manganese (Mn), a key cofactor for ROS-detoxifying enzymes, was significantly upregulated in biofilm cells. Although a multiple Mn-uptake mutant produced biofilms comparable to the wild type, it showed markedly higher susceptibility to H<sub>2</sub>O<sub>2</sub> during and after biofilm formation.

**Conclusions:** We have identified the genes specifically regulated by CsgD or the presence of the biofilm matrix during Salmonella biofilm development. Matrix production triggers a global metabolic shift, ROS accumulation, and induction of oxidative stress responses. Among these, Mn uptake is essential for biofilm cell survival under oxidative challenge, highlighting it as a potential target for biofilm control. These findings support a strategy combining Mn-chelating and oxidizing agents to inhibit Salmonella biofilm formation.



## OP11.1 - ADAPTIVE TRAJECTORIES TO CIPROFLOXACIN RESISTANCE IN A MURINE BIOFILM LUNG INFECTION MODEL

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**Background & Aims:** Chronic biofilm-associated infections pose a significant clinical challenge due to their inherent antibiotic resistance and rapid evolution. Understanding host-pathogen interactions during this process is vital for developing effective interventions. This study aimed to investigate the evolutionary trajectories of *Pseudomonas aeruginosa* under ciprofloxacin (CIP) exposure, focusing on the interplay between genotypic mutations, metabolic rewiring, and host inflammatory responses.

**Methods:** A murine biofilm lung infection model was utilized to study the evolution of antimicrobial resistance over four consecutive passages. To investigate the evolution of *P. aeruginosa*, bacteria are embedded in alginate beads (Day 0) and injected into the lungs of mice (Day 1). Over Days 2–3, mice receive twice-daily treatments of 0.25 mg Ciprofloxacin. On Day 4, the lungs are harvested and homogenized for analysis; surviving colonies from the CIP-treated group are then cultured and re-embedded into fresh beads to infect a new group of mice (next passage). Resistance was characterized through phenotypic MIC testing and whole-genome sequencing. We also integrated longitudinal metabolomic profiling with host response assessments, including histopathological scoring of lung tissue and cytokine measurements using Luminex.

**Results:** The antibiotic exposure accelerated the emergence of resistance, transitioning from an initial mutation-driven tolerance phase to high-level resistance. Sequenced isolates mirrored clinical mechanisms, harboring concurrent mutations in *nfxB* and *gyrA*. Metabolomic profiling revealed a "metabolic canalization" toward a distinct resistant-state fingerprint. Principal Component Analysis identified the *nfxB* gene as the primary driver of metabolic variation (49.1%), significantly depleting central carbon sources like glucose and tyrosine. Furthermore, high-level CIP resistance correlated with collateral sensitivity to tobramycin and aztreonam. While untreated mice exhibited severe neutrophilic inflammation and goblet cell hyperplasia—resembling pathological changes in patients—CIP treatment successfully reduced histopathological lung scores by the fourth passage, despite early spikes in inflammatory cytokines.

**Conclusions:** This in vivo model effectively focuses on the complexity of bacterial adaptive evolution, demonstrating that CIP resistance is connected with a coordinated rewiring of nutrient utilization pathways. The identification of collateral sensitivity highlights a potential evolutionary trade-off that can be exploited. This platform serves as a robust translational tool for developing collateral-sensitivity-informed therapeutic strategies against chronic lung biofilms.



## OP11.2 - SORBED HOST PROTEINS MEDIATE NEUTROPHIL ADHESION, MOTILITY, AND DISCOVERY OF STAPHYLOCOCCUS AUREUS ON AN ABIOTIC SURFACE

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**Background & Aims:** The vulnerability of implanted medical devices to biofilm infection may be in part due to defects in innate immunity around abiotic surfaces. These defects could include delayed recruitment of neutrophils to the surface of an implant and compromised function of neutrophils on that abiotic surface. The aim of this work was to explore the latter hypothesis, specifically that biologically-active sorbed host proteins are required for neutrophil adhesion, motility, and discovery of bacteria on abiotic surfaces.

**Methods:** Video microscopy was applied to directly image the in vitro contest between bacteria (green fluorescent protein-tagged *S. aureus*) attached to an abiotic surface and human neutrophils. Neutrophil functions including adherence, motility (random, chemotactic, and swarming), bacterial discovery, and killing were visualized and quantified.

**Results:** On a surface coated in normal human serum, bacteria were readily cleared if neutrophils discovered the bacterial aggregate before it grew too large. Heat-inactivation of serum abolished neutrophil surface adhesion, motility, and discovery of *S. aureus*, resulting in bacterial persistence. Inhibition of the neutrophil adhesin MAC-1, a receptor that recognizes heat-labile protein ligands including fibrinogen and complement iC3b, reduced motility leading to delayed discovery and persistence of bacteria. Attachment and subsequent neutrophil motility were impaired in complement C3-depleted serum but not cobra venom factor-depleted serum, suggesting that attachment to a serum-coated surface is mediated by complement C3 or its degradation product iC3b. In the absence of complement, surface adsorbed fibrinogen restored adhesion and motility, but a defect in *S. aureus* discovery remained. In some instances, rapid, aggressive neutrophil swarming was observed that resulted in fragmentation and phagocytosis of even relatively large bacterial aggregates.

**Conclusions:** Sorbed host proteins and complement activity play a critical role in mediating effective neutrophil function on an implant surface, pointing to possible immunotherapeutic approaches for preventing medical device infection.



### OP11.3 - ESTABLISHMENT OF A CHRONIC ORTHOPAEDIC INFECTION MODEL WITH MATURE BIOFILM FORMATION USING AN IN VITRO-IN VIVO HYBRID APPROACH

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**Background & Aims:** Chronic biomaterial-associated infections (BAI), particularly those involving biofilm formation, represent a significant clinical challenge. Few *in vivo* models involving mature biofilm formation are available. Mostly based on vertebrates, they are often costly and subject to strict animal welfare regulations. Most simple non-vertebrates *in vivo* models, such as *Galleria mellonella*, have rarely been investigated to study chronic infections due to their limited lifetime. Considering this point, this study aims to establish an *in vitro-in vivo* approach. Static and dynamic flow models were compared to form first strong *in vitro* *S. aureus* biofilms on implants prior to implantation into *G. mellonella* larvae.

**Methods:** Three orthopaedic-related *S. aureus* strains were selected to form biofilms on the surface of Kirschner wires (K-wires) over an incubation period of up to 7 days using either a static (microplate model) or a dynamic flow system (CBR; BioSurface Technologies). After biofilm maturation periods of 1 and 7 days, the implants were washed and then used to: (i) characterize biofilms (quantitative cultures, SEM, and gene expression of biofilm markers), (ii) assess *in vitro* rifampicin susceptibility (EUCAST), and (iii) introduce the implants into *G. mellonella* larvae for an additional 5 days. Rifampicin administration to larvae (80 µg/g) was assessed as a surrogate marker for biofilm maturation using Kaplan-Meier analysis.

**Results:** The data demonstrated that dynamic flow maturation in the CBR for 7 days, followed by implantation into *G. mellonella* larvae, led to mature biofilm formation with markedly decreased rifampicin effectiveness. Furthermore, unlike biofilms formed under static conditions, dynamic flow conditions significantly increased biofilm formation after 7 days. This approach resulted in a 2-4 log<sub>10</sub> CFU increase on K-wires and the formation of thicker biofilms, partly due to enhanced secretion of extracellular matrix components, prior to introduction into the larvae.

**Conclusions:** Overall, we established an *in vitro-in vivo* hybrid method to mimic chronic orthopaedic implant infection with mature biofilm formation. The use of a high-shear flow device to initiate strong biofilm formation and maturation on orthopaedic device surfaces prior to introduction into larvae proved essential. This high-throughput and cost-effective method could contribute to the assessment of new treatment strategies targeting chronic biofilm infections.



## OP11.4 - THE PUTATIVE AHL RECEPTOR SDIA AS A REPRESSOR OF BIOFILM FORMATION, PHAGOCYTOSIS AND INTRACELLULAR SURVIVAL IN KLEBSIELLA PNEUMONIAE

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**Background & Aims:** *Klebsiella pneumoniae* is a significant opportunistic pathogen, and the rise of multidrug-resistant strains necessitates new therapeutic strategies targeting virulence. In *Escherichia coli*, the quorum-sensing receptor SdiA has been implicated in regulating key infection processes<sup>1</sup>. However, the role in *K. pneumoniae* was barely studied. This study aimed to elucidate the role of SdiA in the pathogenesis of *K. pneumoniae*, focusing on its impact on biofilm formation, capsule production, and macrophage-interaction.

**Methods:** *sdiA* deletion mutant ( $\Delta sdiA$ ) was generated in a clinical (KLEB-33) and collection strain (ATCC 13883<sup>T</sup>) using a CRISPR-Cas9 system<sup>2</sup>. The role of SdiA in biofilm formation was assessed with the Rolling Biofilm Bioreactor (RBB) model<sup>3</sup>. Phagocyte-interaction was tackled using immortalised murine bone-marrow derived macrophages (iBMDMs) to evaluate adhesion, phagocytosis, and intracellular survival dynamics using plate-counting and confocal imaging (Leica Stellaris 5). Macrophage response was evaluated with cytokine (mIL-1 $\beta$ , mTNF $\alpha$ , mCXCL10), Reactive Oxygen Species (ROS), and viability (Neutral Red) quantification. Moreover, capsular polysaccharide (uronic acid quantification) and *E. coli* predation was evaluated in bacterial strains used.

**Results:** Deletion of *sdiA* resulted in a significant decrease in uronic acid content, suggesting that SdiA positively regulates capsule production as suggested previous reports<sup>2</sup>. The diminished capsule production correlated with enhanced adhesion capabilities, as  $\Delta sdiA$  strains showed significant higher biofilm formation in RBB model, as well as higher adhesion and uptake/phagocytosis by iBMDMs compared to wild-type strains. Moreover, the *sdiA*-mutants exhibited enhanced intracellular survival within macrophages. Despite this, macrophage response was not impaired: in  $\Delta sdiA$ -infected macrophages a higher ROS and cytokine production was detected, although viability recorded was lower at the end of infection. Furthermore, *sdiA* -mutant strains did not kill *E. coli* preys, contrary of wild-type ones.

**Conclusions:** SdiA represses capsule production, biofilm formation, and adhesion, phagocytosis, and intracellular survival in iBMDMs. However, *sdiA*-lacking strains does not counteract host defences, indicating that the observed enhanced intracellular survival could be due to initial bacterial overload. The study highlights a dual and complex role for SdiA in modulating virulence as well as the interaction between *K. pneumoniae* and phagocytic cells.

References: (1) Mayer et al., 2023. doi:10.1093/femsre/fuad031. (2) Silva-Bea et al., 2025. doi:10.3389/fcimb.2025.1562402. (3) Romero et al., 2022. doi: 10.1111/1462-2920.15985.



## OP12.1 - A WEARABLE INTRACORNEAL METALLOENZYME-MICRONEEDLE PLATFORM EMBEDDED WITH AN INBUILT ANTIBACTERIAL FUNCTION THROUGH INORGANIC DNASE FOR THE TREATMENT OF KERATITIS INFECTIONS

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**Background & Aims:** Bacterial Keratitis (BK) is a leading cause of vision-threatening disease and is difficult to treat due to antibiotic resistance and high recurrence rates. BK caused by *Pseudomonas aeruginosa* is particularly severe, as it is highly invasive and can cause devastating damage to the entire cornea within a very short period, thereby attracting greater clinical attention. Additionally, dense 3D biofilm formation protects bacteria from therapeutics and the immune system, and the resulting excessive inflammation further complicates treatment. All currently available therapeutic approaches allow only 1-3% of the applied total dose to permeate the cornea, are prone to antimicrobial resistance (AMR), and have a short pre-corneal residence time. To overcome these limitations of current BK therapy, and inspired by the 'metalloantibiotics' concept, we aimed to develop a novel antibiotic-integrated inorganic deoxyribonuclease to address both AMR and corneal damage simultaneously.

**Methods:** We first engineered a novel metalloantibiotic complex with deoxyribonuclease (DNase)-mimetic activity. This complex was synthesized by chemical conjugation, characterized for structure and physicochemical properties (via PXRD, XPS, FTIR, TGA, DLS), and integrated into a microneedle (MN) patch via a PDMS- based micromolding approach for precise intracorneal delivery. Its efficacy was then evaluated through in vitro assays measuring antimicrobial activity, biofilm disruption (via CFU counts, CLSM, growth kinetics), and DNase-mimicking activity. Finally, biocompatibility and immunomodulatory effects were assessed on human corneal epithelial cells and immune cells using viability assays, CLSM, and RT-qPCR.

**Results:** The innovative metalloenzyme-MN platform demonstrated a multi-mechanistic therapeutic action. The DNase-mimetic metalloantibiotic complex effectively hydrolyzed extracellular DNA (eDNA), destabilizing pre-formed biofilms and exposing embedded bacteria. Concurrently, the released antimicrobial payload efficiently eradicated the liberated pathogens through oxidative stress, cell membrane damage, and immune stimulation. Furthermore, the patch orchestrated a controlled anti-inflammatory response, alleviating local inflammation, mitigating ulceration, and promoting the repair of damaged corneal tissue.

**Conclusions:** This study introduces a transformative intracorneal microneedle platform that directly targets the core patho- physiological triad of BK, *i.e.*, biofilm integrity, bacterial viability, and dysregulated inflammation. By synergistically disrupting biofilms, eradicating infection, and resolving inflammation to accelerate healing, this innovative approach presents a promising strategy to overcome the critical limitations of current BK therapies.



## OP12.2 - N-TERMINAL LIPIDATION BOOSTS ANTIFUNGAL LIPOPEPTIDES WITH ACTIVITY AGAINST MATURE CANDIDA BIOFILMS AND IMPROVED MEMBRANE TARGETING

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**Background & Aims:** Fungal biofilms represent a major barrier in invasive fungal infections, contributing to tolerance to conventional antifungals and persistence on medical devices. Short antimicrobial peptides are promising alternatives but often show limited activity against yeasts and biofilm-embedded cells. Here, we investigated whether N-terminal lipidation enhances the membrane interaction, antibiofilm efficacy, and safety profile of Trem-4 (WKLLRGIVKV), a decapeptide derived from Trematocine-HSK.

**Methods:** Trem-4 was N-terminally functionalized with caprylic (C8) or myristic (C14) fatty acids to yield Cap-T4 and Myr-T4. Antifungal activity was assessed against 60 clinically characterized yeast isolates (*Candida albicans*, *C. glabrata*, *C. parapsilosis*, *Candidozyma auris*, *C. tropicalis*, *C. vulturna* and *Cryptococcus neoformans*) under planktonic and biofilm conditions. Mature 72-h biofilms of selected high biofilm-producing strains were treated at 1× and 5× MIC and evaluated by XTT metabolic assays, scanning electron microscopy (SEM), and Live/Dead fluorescence staining. Peptide-membrane interactions were investigated using tryptophan fluorescence partitioning into zwitterionic (POPC) and anionic (POPC/POPG) large unilamellar vesicles, acrylamide quenching, critical aggregation concentration (CAC) determination (ANS probe), and circular dichroism. Biocompatibility was evaluated by haemolysis, mammalian cell cytotoxicity assays, and *in vivo* tolerability in *Galleria mellonella*.

**Results:** Compared to non-lipidated Trem-4 (inactive against yeasts at tested concentrations), both lipopeptides showed broad antifungal activity across clinically relevant species, including isolates resistant to standard antifungals, with median MICs generally ranging from 8 to 32 µg/mL depending on species. Importantly, both compounds retained activity against mature biofilms, causing pronounced metabolic impairment and structural disruption (biofilm thinning, reduced cohesion, and extracellular matrix disruption by SEM); XTT assays showed 28–50% residual metabolic activity at 1×MIC and near-complete suppression at 5×MIC in selected species. Cap-T4 displayed more consistent inhibitory activity across species, whereas Myr-T4 showed enhanced efficacy against selected isolates, including *C. auris*. Lipidation markedly increased membrane affinity versus Trem-4; chain length shaped aggregation (lower CAC for C14) and selectivity profiles. Cytotoxicity and haemolysis were concentration-dependent and more evident for Myr-T4, while both lipopeptides were well tolerated *in vivo*.

**Conclusions:** N-terminal lipidation converts a short peptide into a membrane-active antifungal lipopeptide with measurable efficacy against mature fungal biofilms. These results support lipid-modified peptides as candidates for emerging antibiofilm strategies against drug-resistant yeasts.



## OP12.3 - DEFYING GRAVITY: COLD PLASMA TECHNOLOGY TO DEFEAT BIOFILMS IN SPACE

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**Background & Aims:** 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 oxygen and nitrogen species, has shown promise as an antibiotic-free antimicrobial. Therefore, we hypothesise that PAW retains its antibiofilm activity after irradiation, and is equally effective against biofilms grown in microgravity vs static conditions. We aim to evaluate the efficacy of irradiated PAW against *Staphylococcus aureus* biofilms grown terrestrially vs in simulated microgravity.

**Methods:** 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 nitric acid ( $\text{HNO}_3$ ) as a pH control. Physicochemical properties including pH, conductivity and oxidative-reductive potential (ORP) were tracked over 5 months. Biofilms were treated with irradiated PAW for 5 min, and antibiofilm activity was assessed by colony-forming unit (CFU/mL) counts ( $n \geq 3$ ). Statistical analysis: two-way ANOVA and Šídák's multiple comparison tests ( $p < 0.05$ ).

**Results:** PAW showed no statistically significant differences between radiation conditions for pH, conductivity or ORP. Terrestrially-grown biofilms experienced 2-log reductions after  $\text{HNO}_3$  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 tolerant, with 1-log reductions after  $\text{HNO}_3$  or PAW treatment as compared to water treatment ( $p < 0.05$ ).

**Conclusions:** Overall, irradiated PAW effectively reduced terrestrial biofilms without changes in physicochemical properties, while microgravity-grown biofilms showed enhanced tolerance to  $\text{HNO}_3$  and PAW treatment. This underscores biofilm contamination as a key challenge for long-duration spaceflight, necessitating further research to curb biofilm-related infections in space. Although PAW demonstrates promise as a space-compatible antimicrobial, further optimisation is necessary before it can be fully considered for use in space environments. This includes comparing PAW efficacy against existing space-approved antimicrobials.



## OP12.4 - TARGETED PHAGE THERAPY EFFECTIVELY DISRUPTS KLEBSIELLA PNEUMONIAE BIOFILMS: IMPLICATIONS FOR WOUND INFECTION TREATMENT

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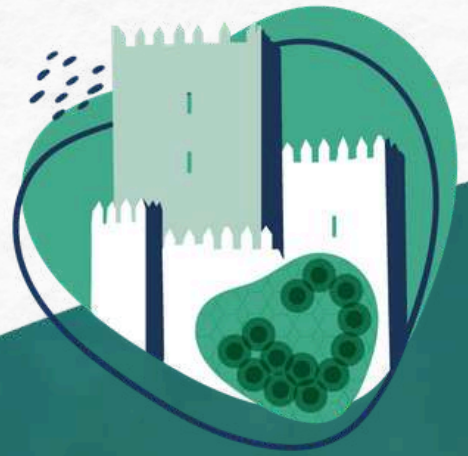
**Background & Aims:** Biofilms are notoriously tolerant to antibiotics, hampering the cure of chronic wound infections in both military and civilian settings, demanding alternative therapeutics. To explore phage therapy as a non-antibiotic alternative, one of the aims of 'RESILIENCE' European Defense Ministry consortium is to evaluate three clinically relevant *Klebsiella pneumoniae* (Kp) strains (K1-serotype NTUH K2044, K2-serotype CIP 52145, K2-serotype ATCC 43816) and their isogenic capsule deficient mutants for biofilm formation, virulence, and susceptibility to a panel of lytic phages in *in vitro* biofilm and *in vivo* models.

**Methods:** All Kp strains were engineered to carry a chromosomal *luxABCDE* reporter (at Tn7 site) for real-time bioluminescence tracking. Biofilm biomass was quantified by Crystal Violet assay in three media: LB, minimal M63B1 + 0.4 % glucose, and Lubbock Chronic Wound Biofilm Medium. Virulence was measured in *Galleria mellonella* larvae. Our phage panel includes K2-specific phages (VICEL, fRuKpn01, KLEO31, KLEO170), K1-specific phage KLEO1 and the non-capsulated targeting phage P015. Mature biofilms were exposed to our phage panel at defined multiplicities of infection (MOI); residual viability of biofilm-dwelling bacteria was assessed via fluorescein diacetate conversion. *In vivo* phage efficacy against Kp infection was evaluated by bioluminescence tracking and colony-forming unit (CFU) counts in larvae model till 72 hours.

**Results:** The strains NTUH-K2044 and 43816 formed dense biofilms in all media, whereas CIP 52145 was modest. Absence of capsule enhanced biofilm only in NTUH-K2044. In larvae, NTUH-K2044 and 43816 were the most virulent, with attenuation in absence of capsule. Phage treatments disrupted *in vitro* mature biofilms in a strain- and MOI-dependent fashion, and was globally efficient against the Kp strains tested. And the reduced viability of biofilm-dwelling bacteria correlated with the biofilm disruption observed. In larvae model, phage- treated groups showed a progressive CFU decline for every strain-phage pairing tested, throughout the 72 hour observation period.

**Conclusions:** Targeted phages efficiently fight both capsulated and capsule-deficient Kp biofilms and reduce *in vivo* bacterial burden. These data validate phage therapy as a viable, antibiotic-free, strategy for treating recalcitrant Kp wound infections, aligning with RESILIENCE consortium's goal to deliver rapid, frugal treatments for conflict zone casualties.

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PRESENTATIONS



## FP1 - ADVANCING BIOFILM INNOVATION: BUILDING CONSENSUS IN REGULATORY DECISION MAKING

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**Background & Aims:** Regulatory science provides the frameworks and evidentiary standards that enable authorities to determine whether antimicrobial products, devices and materials are safe, effective and suitable for market approval. These frameworks underpin label claims and risk-benefit decisions that protect public health and the environment. However, most regulatory microbiology approaches were developed around planktonic systems and simplified assays. They fail to capture the structural complexity, tolerance mechanisms, heterogeneity and environmental resilience of biofilms, or reflect advances in biofilm science and modern methodologies to study them. This mismatch between contemporary biofilm science and regulatory evidence requirements limits credible antibiofilm claims and slows innovation. The European COST Action CA23152, RegulatoryToolBox, was established to close this gap by building consensus on evidence based approaches that support robust, harmonised evaluation of antibiofilm claims across Europe.

**Methods:** RegulatoryToolBox brings together academic researchers, clinicians, industry stakeholders and regulatory experts across Europe to update the equipment, models and methods used by regulatory, industrial and clinical stakeholders for improved decision making. Methodologies are critically appraised against performance criteria, application relevance and decision-making utility. Cross-sector case analyses and structured engagement identify bottlenecks, clarify evidence expectations and prioritise areas requiring methodological alignment.

**Results:** As a newly established network, RegulatoryToolBox is generating the foundational analyses required to support consensus development. Early mapping highlights substantial gaps in both the understanding of biofilms and the provision of appropriate guidelines. These gaps create ongoing challenges in interpreting biofilm data and in reliance on methods not fit for purpose, particularly for non typical or emerging antibiofilm technologies. Stakeholder consultations also reveal broader regulatory gaps, including limited flexibility to adapt evidence requirements to the intended application context and insufficient recognition of novel mechanisms or modes of action. Collectively, these insights underscore the need for more adaptive, scientifically grounded approaches and are informing the development of a structured evaluation framework to guide subsequent coordinated activities.

**Conclusions:** RegulatoryToolBox represents a shift towards proactive, evidence-led biofilm regulatory science. By defining what credible, decision-ready biofilm evidence should look like, the Action is laying the groundwork for lasting alignment between scientific innovation and regulatory decision making across Europe.



## FP2 - NITRATE RESPIRATION REGULATES ESCHERICHIA COLI BIOFILM MORPHOGENESIS THROUGH A REDOX-RESPONSIVE DIGUANYLATE CYCLASE

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**Background & Aims:** The biofilm lifestyle is tightly linked to environmental adaptation. Nitrate respiration has been shown to regulate biofilm formation strain-specifically in commensal and pathogenic *Escherichia coli* strains. Here, we dissect the molecular basis of nitrate respiration-induced biofilm turnover in Enterotoxigenic *E. coli* (ETEC), with the aim of identifying key mediators and defining their role in biofilm morphogenesis.

**Methods:** Two ETEC strains exhibiting opposite biofilm responses to exogenous nitrate supplementation on Congo Red-supplemented agar, that is, biofilm downshift versus upshift, were analyzed. Whole-genome transcriptomic analyses were performed on wild-type (WT) and nitrate respiration mutants grown with or without nitrate to identify differentially expressed genes. Nitrate respiration-deficient isogenic mutants and additional targeted mutants were generated by recombineering. The role of the diguanylate cyclase DgcJ was examined using site-directed mutagenesis, immunoblotting, protein-based assays, and phenotypic analyses of motility, biofilm formation, and nitro-oxidative stress.

**Results:** Transcripts encoding a single diguanylate cyclase, DgcJ, were significantly dysregulated in both ETEC strains in response to nitrate. In nitrate respiration-deficient mutants, nitrate supplementation resulted in markedly fewer transcriptional changes compared to WT strains, defining a nitrate respiration-dependent regulon. Genetic analyses demonstrated that DgcJ is a central regulator of colony biofilm morphogenesis in both isolates. A conserved pair of cysteines forming a redox switch and tryptophan residues within the GAPES1 sensor domain of DgcJ were found to control diguanylate cyclase activity, swimming motility, and biofilm formation. The requirement for both oxygen and nitrate for phenotype development suggests nitro-oxidative stress as a mediator.

**Conclusions:** These findings identify DgcJ as a redox-responsive enzyme linking nitrate respiration to biofilm morphogenesis in ETEC. DgcJ integrates periplasmic stimuli via its GAPES1 domain to modulate cyclic di-GMP-dependent behaviors, providing mechanistic insight into respiration-driven biofilm regulation and bacterial adaptation to perturbed redox homeostasis. Additional mechanisms further regulate how ETEC strains respond differently to nitrate.



## FP3 - EXPOSURE TO EMERGING CHEMICALS RESHAPES BIOFILMS AND PROMOTES ANTIBIOTIC TOLERANCE

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**Background & Aims:** Chlorination is widely used to control microbial contamination in hospital drinking water (DW) systems. However, chlorine can react with organic contaminants to form potentially harmful disinfection by-products (DBPs). Parabens enter hospital wastewater through personal care products and pharmaceutical residues and react with chlorine to form chlorinated compounds. These products persist in hospital DW and may affect patient health due to their vulnerability and the presence of persistent plumbing biofilms. This study aimed to evaluate the impact of methylparaben (MP) and its chlorinated compounds: mono-chlorinated methylparaben (3-Cl-MP) and di-chlorinated methylparaben (3,5-diCl-MP) on DW biofilms.

**Methods:** Dual-species biofilms of *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia* were grown in the presence of MP, 3-Cl-MP, and 3,5-diCl-MP at 15 µg/L for 7 days. Then, the effects of these compounds on bacterial characteristics (culturability, density, viability), biofilm structure, and composition of extracellular polysaccharide substances (EPS) were assessed. The impact of MP, 3-Cl-MP, and 3,5-diCl-MP on bacterial tolerance to chlorine and antibiotics was also evaluated.

**Results:** Exposure to 3-Cl-MP and 3,5-diCl-MP increased the number of viable but not culturable (VBNC) cells of *A. calcoaceticus* by 3-fold and 5-fold, respectively. Exposure to 3-Cl-MP also resulted in an increase in the VBNC levels of the overall dual-species biofilms and a 10 µm increase in biofilm thickness. The polysaccharide content was higher for 3-Cl-MP and 3,5-diCl-MP-exposed biofilms. Exposure to MP increased biofilm tolerance to chlorine (5 mg/L), with lower log CFU/cm<sup>2</sup> reductions obtained ( $0.51 \pm 0.09$ ) in relation to the control ( $0.66 \pm 0.12$ ). However, biofilms exposed to 3-Cl-MP showed increased susceptibility to chlorine, with higher log CFU reductions for *A. calcoaceticus* ( $1.26 \pm 0.07$ ), *S. maltophilia* ( $1.21 \pm 0.14$ ), and overall dual-species biofilms ( $1.17 \pm 0.11$ ). Similarly, increased reductions in total cells were obtained for 3-Cl-MP-exposed *A. calcoaceticus*, and for the overall dual-species biofilms after 3,5-diCl-MP exposure. Curiously, 3,5-diCl-MP exposure increased the antibiotic tolerance of *S. maltophilia* to minocycline and trimethoprim-sulfamethoxazole.

**Conclusions:** MP and its chlorinated compounds can significantly alter bacterial physiology and biofilm characteristics in hospital DW systems. While MP increased bacterial tolerance to disinfection, its chlorinated compounds enhanced VBNC formation, altered EPS composition, and increased antibiotic tolerance in *S. maltophilia*.



## FP4 - ANTIGEN-ANTIBODY-BASED EARLY WARNING SYSTEM FOR DETECTING EPS BIOMARKERS DURING BIOFILM INITIATION

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**Background & Aims:** Biofilms are structured microbial communities embedded within a self-produced extracellular matrix of polysaccharides, proteins, lipids, and extracellular DNA. Once established, they exhibit high tolerance to antimicrobials and immune responses, making eradication difficult. Biofilms impose a major economic burden, contributing to an estimated \$2.5 trillion annually in global corrosion costs, with ~20% linked to microbiologically influenced corrosion (MIC) (NACE International), and \$28–45 billion per year in U.S. healthcare-associated infections (Centers for Disease Control and Prevention). A fundamental challenge in biofilm detection is the lack of a consistent relationship between planktonic growth dynamics and the initiation of biofilm maturation. Conventional monitoring methods rely on optical density measurements or colony-forming unit counts, which reflect bacterial proliferation but provide no direct insight into extracellular matrix development. Consequently, biofilm mitigation strategies remain reactive rather than predictive, resulting in excessive biocide use, incomplete removal, and resistance escalation. This invention addresses this limitation by introducing a biochemical sensing strategy based on the detection of modified protein adducts formed as metabolic end products and incorporated into the extracellular polymeric substance (EPS) matrix. These molecular modifications accumulate during biofilm development and provide a matrix-associated signal that is independent of planktonic growth. Monitoring the dynamics of these EPS-associated modified protein adducts enables predictive detection of biofilm formation prior to structural maturation.

**Methods:** A competitive ELISA-based assay was developed and optimized to quantify modified protein adducts associated with developing biofilms. The assay monitors competitive binding of sample-derived adducts against immobilized protein-adduct substrates, enabling indirect quantification of matrix-associated molecular modifications. Signal inhibition correlated with biofilm progression, confirming measurable changes in adduct accumulation during matrix development. In parallel, confocal laser scanning microscopy provided spatial validation. Fluorescently labeled detection antibodies were used to localize modified protein adducts within the biofilm extracellular matrix. Co-staining with plasma membrane markers confirmed that the detected signal was predominantly extracellular and matrix-associated rather than intracellular, supporting temporal linkage between adduct accumulation and biofilm maturation. A biofilm inhibition strategy targeting pathways responsible for formation of these protein adducts was further evaluated at laboratory scale. Preliminary in vitro results demonstrated that suppression of adduct formation correlated with reduced matrix development and altered biofilm architecture, supporting the mechanistic basis of this early biofilm detection approach.

**Results:** Protein adduct levels quantified by competitive ELISA were measured in parallel with the biofilm growth curve. A strong linear correlation was observed between protein adduct concentration and crystal violet (CV) staining intensity, indicating proportional association with biofilm biomass accumulation. When plotted against the growth curve, protein adduct concentrations demonstrated an exponential increase coinciding with the onset of early biofilm formation. In contrast, planktonic cultures maintained consistently low protein adduct concentrations, with no comparable exponential rise, indicating that adduct accumulation is matrix-associated rather than growth-dependent. Pharmacological inhibition of pathways involved in metabolic protein adduct formation resulted in significantly reduced adduct concentrations. Importantly, timing of inhibitor administration was critical: intervention during the early exponential rise in adduct levels effectively suppressed biofilm formation without affecting planktonic growth. These findings support the role of protein adduct accumulation as an early predictive marker of biofilm initiation and as a target for temporally optimized intervention strategies.

**Conclusions:** These findings demonstrate that accumulation of matrix-associated protein adducts serves as an early and predictive biochemical marker of biofilm initiation, independent of planktonic growth. Their exponential rise precedes structural maturation and correlates directly with biomass formation. Targeted inhibition of adduct-forming pathways, when applied at the appropriate temporal window, suppresses biofilm development without affecting planktonic cells. This approach represents a universal strategy for early biofilm detection across microbial communities. The system is currently being tested in a membrane treatment unit to develop a predictive model for real-time monitoring and intervention, highlighting its potential for both industrial and environmental applications.



## FP5 - ERADICATION OF LISTERIA MONOCYTOGENES BIOFILM USING THE COMBINED TREATMENT WITH PHYTOCHEMICALS, BLUE LIGHT AND SONICATION

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**Background & Aims:** Innovative biofilm treatment methods using light irradiation and ultrasound are gaining increased attention, where certain phytochemicals may serve as effective photo- and sono-sensitizers. When combined, these methods may sufficiently eliminate preformed biofilms, particularly against *Listeria monocytogenes*, one of the leading causes of death from foodborne infections. After recognizing the effects of phytochemicals and individual treatments on single strains, this study further investigated a combined approach utilizing three phytochemicals: curcumin, quercetin, and capsaicin, along with blue light (BL) and ultrasonication (US) to eliminate multi-strain biofilms of *L. monocytogenes*.

**Methods:** Three strains were selected based on strong biofilm-forming capabilities and combined to form a cocktail for biofilm growth in a DK20 Rotating Disk Biofilm Reactor (BioSurface Technologies). The biofilms were exposed to BL using a Honle® LED Cube 100 HP IC system, delivering 25 J/cm<sup>2</sup> and 50 J/cm<sup>2</sup>. Biofilms were subjected to US using a VialTweeter paired with a UP200St unit (Hielscher), providing an ultrasonic intensity of 10 watts per biofilm for 30 seconds. Treatments were applied sequentially, 25 J/cm<sup>2</sup>→US and 50 J/cm<sup>2</sup>→US, with each of the three phytochemicals at subinhibitory concentrations.

**Results:** Biofilm cell counts ranged from 7.59 log CFU/cm<sup>2</sup> for untreated biofilms to 3.20 log CFU/cm<sup>2</sup> for treated biofilms. BL reduced biofilm levels by 3.62% at 25 J/cm<sup>2</sup> and by 16.78% at 50 J/cm<sup>2</sup>. Curcumin further increased the reduction rates to 8.84% and 77.55% (p<0.05). Adding another hurdle, significantly improved biofilm reduction to 35.37% at 25 J/cm<sup>2</sup> and nearly eliminated biofilms at 50 J/cm<sup>2</sup>. Quercetin enhanced the effectiveness of BL by 15% across both BL doses. When combined with the US, the biofilm reductions increased to 35.15% and 53.51%, respectively. The combination of hurdles with a lower BL dose differed significantly from 25 J/cm<sup>2</sup> alone (p<0.05). Capsaicin did not enhance biofilm elimination at any dose (p>0.05). However, when combined with the US, biofilm reduction increased by 20%, with a significant difference noted at 25 J/cm<sup>2</sup>.

**Conclusions:** The investigation reveals previously unrecognized properties of phytochemicals as effective photo- and/or sono-sensitizers against biofilms of *L. monocytogenes*, providing new opportunities for treatment options. This research was funded by the National Science Centre, Poland [Grant number 2022/45/B/NZ9/00299 (OPUS-23)].



## FP6 - EVALUATION OF AMOXICILLIN AND CIPROFLOXACIN ACTIVITIES AGAINST ENTEROCOCCUS FAECALIS BIOFILMS IN CATHETER- ASSOCIATED URINARY TRACT INFECTIONS USING A DYNAMIC IN VITRO PHARMACOKINETIC-PHARMACODYNAMIC FLOW MODEL

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**Background & Aims:** Catheter-associated urinary tract infections (CAUTIs) are among the most common healthcare-associated infections. Among the major uropathogens, *Enterococcus faecalis* is one of the most frequently implicated species in CAUTIs due to its strong ability to form biofilms on urinary catheter surfaces, which promotes persistent colonization and reduces susceptibility to antimicrobial therapy. However, current antimicrobial dosing strategies are primarily based on planktonic susceptibility testing, limiting their relevance to biofilm-associated infections. To bridge this gap, we developed a dynamic *in vitro* pharmacokinetic-pharmacodynamic (PK-PD) flow model simulating urinary PK to evaluate the activity of amoxicillin and ciprofloxacin against *E. faecalis* biofilms.

**Methods:** Biofilms of *Enterococcus faecalis* ATCC 29212 and strain 54 were pre-formed on catheter segments. Mature biofilms were subsequently exposed in a dynamic *in vitro* PK-PD model to simulated urinary dosing regimens of amoxicillin (AMX; 500 mg q12h, 3 days) or ciprofloxacin (CIP; 500 mg q24h, 3 days), as well as to constant antibiotic concentrations over a 3-day period. Bacterial viability within the biofilms, assessed by colony-forming units (CFU), and biofilm biomass, measured using crystal violet staining, were evaluated at different time points. Finally, the antibacterial effects observed under simulated dosing regimens were compared with those under constant concentration conditions.

**Results:** After 3 days of antimicrobial exposure, the simulated ciprofloxacin regimen produced the greatest reductions in both bacterial viability and biofilm biomass, significantly exceeding the effects observed with constant CIP exposure and with both amoxicillin regimens in both strains. Although the simulated urinary AMX profile demonstrated greater bactericidal activity compared with constant AMX exposure, both AMX regimens achieved only modest reductions in biofilm biomass. Notably, constant concentration of AMX exposure was associated with enhanced biofilm formation.

**Conclusions:** These findings demonstrate that the antimicrobial efficacy against *E. faecalis* biofilms is highly dependent on both the chosen agent and the dosing regimen. Dynamic *in vitro* PK-PD flow models offer substantial translational value and represent a promising approach for optimizing antimicrobial dosing strategies, as well as for supporting the development of novel anti-biofilm interventions for CAUTIs.



## FP7 - SYNERGISTIC EFFECT OF CEFTOLOZANE/TAZOBACTAM AND PHAGES IN BIOFILM-ASSOCIATED RESPIRATORY INFECTIONS CAUSED BY CARBAPENEM-RESISTANT *PSEUDOMONAS AERUGINOSA*

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**Background & Aims:** This study evaluated the synergism between ceftolozane/tazobactam (CT) and phages, *in vitro* and *in vivo*, in biofilm-associated respiratory infections caused by carbapenem-resistant *Pseudomonas aeruginosa* (CRPA).

**Methods:** For the *in vitro* study, 6 phage cocktails (PCKs; 10<sup>8</sup> PFU/mL of 3 lytic phages with Efficiency of Plating ≥ 0.1) were designed and tested on 48-h endotracheal tube (ETT) biofilms formed by 3 CRPA strains. PCKs or CT (5 µg/mL) were administered alone or in combination q12h for up to 96h. Viable counts and resistance to CT and phages were assessed q24h. The PCK that reduced *in vitro* biofilms was applied in mechanically ventilated pigs with severe ventilator-associated pneumonia caused by VAP2 (MIC 4 µg/mL) and randomized to receive: Saline (NaCl 0.9% q12h), or CT (50 mg/kg; q8h), or PCK (10<sup>9</sup> PFU/mL; intravenously and by nebulisation q12h), or PCK with CT. Bacterial load in tracheal secretions (TS) was measured q12h, and *in vivo* ETT biofilms were assessed after 72 h.

**Results:** *In vitro*, PCKs or CT alone induced short-term biofilm reduction, however the effect was not sustained. PCKs-CT treatment showed synergistic activity against all strains. Biofilm reduction ranged from 2.42 to 8 log<sub>10</sub> beyond CT alone. Despite the emergence of resistance to CT and to phages during monotherapy, susceptibility to both persisted under combined PCKs-CT treatment. *In vivo*, combined PCK with CT achieved highest reduction of viable CRPA counts in TS and ETT by ≥2 logs.

**Conclusions:** Combined PCK-CT therapy delays the emergence of antimicrobial resistance and achieves greater CRPA reduction in secretions and ETT biofilms compared with monotherapy with either agent.



## FP8 - EFFECT OF SILVER ON THE SURFACE PROPERTIES OF ZRN/AG FOR USE IN PIN TRACT INFECTIONS AND THE IMPACT OF A BLOOD CONDITIONING FILM ON ANTIMICROBIAL EFFICACY

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**Background & Aims:** Medical devices such fixation pins may develop an infection at the surgical site. Following insertion, a medical device immediately becomes coated by a conditioning film, before bacteria bind to a surface which can affect the antimicrobial efficacy of the coating. The aim of this work was to determine the effect that adding silver to a ZrN coating had on the surface properties. In addition, the addition of a blood conditioning film was used to determine its effect on antimicrobial efficacy.

**Methods:** Zirconium nitride/silver (ZrN/Ag) coatings were characterised for their surface tribological properties (Thrust washer testing, Scanning Electron Microscopy), topography (Atomic Force Microscopy), chemistry (Energy Dispersive X-Ray Spectroscopy), physicochemistry (dynamic contact angle), changes to the bacteria (Microbial Adhesion to Hydrocarbons) and antimicrobial efficacy (respiratory activity, ISO 22196, differential staining and retention assays) using *Staphylococcus aureus* and *Staphylococcus epidermidis*, in the absence and presence of a blood conditioning film.

**Results:** The conditioning film altered the microtopography, chemistry and physicochemistry of the surfaces. In addition, the conditioning film reduced the microbial affinity towards the hydrocarbons for both microorganisms. The blood conditioning film reduced the antimicrobial efficacy of the Ti-ZrN/Ag coatings. However, it also reduced the number of retained bacteria.

**Conclusions:** This study suggested that the presence of a defined blood conditioning film reduced the antimicrobial efficacy of the coatings. However, it also resulted in surfaces with anti-adhesive properties and this could potentially lead to a reduction in bacterial retention and risk of infection.



## FP9 - MICROICS DEMONSTRATES THE PREDICTIVE VALUE OF BIOFILM STRUCTURE

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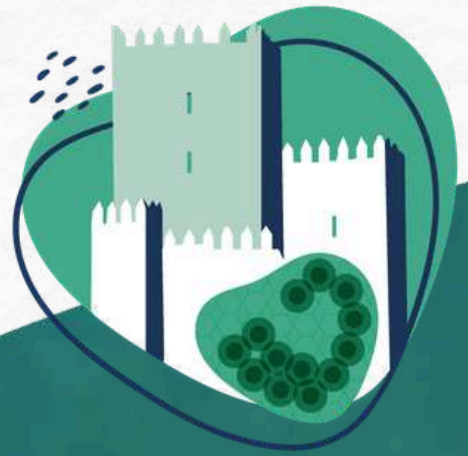
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**Background & Aims:** Microbial communities in biofilms adopt highly structured three-dimensional (3D) structures according to the various microenvironments within the biofilms. In *Listeria monocytogenes*, biofilm-associated persistence represents a major challenge for food safety, as contamination and outbreaks have increased across the European Union over the past two decades. Biofilm-forming capacity showed no clear relationship with virulence traits or genetic similarity, highlighting biofilm formation as a complex, multifactorial phenotype. Previous studies have shown pronounced strain-dependent differences in biofilm architecture. The aim of this study was to determine whether biofilm structure alone can be used to identify strains and infer biologically relevant traits to support the development of innovative biofilm control strategies.

**Methods:** We developed MicroICS, an open-source machine learning pipeline that automatically extracts quantitative structural features from 3D biofilm images. Biofilms formed by eight epidemiologically relevant *L. monocytogenes* strains were imaged using confocal laser scanning microscopy. Over 2,700 quantitative structural descriptors capturing biofilm complexity, density, and spatial organisation were extracted from 3D image stacks. An optimized random forest classifier, refined through hyperparameter tuning and data augmentation, was used for image classification. Feature importance was assessed with the random forest model. The approach was further evaluated by analysing biofilms from hypervirulent versus non- hypervirulent clones and biofilms structurally altered by selected food extracts.

**Results:** MicroICS successfully distinguished biofilms formed by the eight *L. monocytogenes* strains using image-derived features alone. The optimised classifier outperformed human experts in strain identification. Fractal dimension, reflecting biofilm structural complexity, and void fraction, representing biofilm density, were identified as key predictive features, enabling biological interpretation of the model. Extending MicroICS to traits such as hypervirulence identified structural differences, revealing dataset trends, structural drivers, and strain-specific patterns. Application to food extract-treated biofilms demonstrated the method's utility in revealing structural responses to environmental perturbations, while also highlighting interpretative limitations.

**Conclusions:** MicroICS demonstrates that 3D biofilm structure contains predictive information on strain identity and biologically relevant traits. This interpretable, image-based approach provides a powerful tool for biofilm research and may support future strategies to better understand and combat biofilm-related infections in food and clinical settings.



# 11

## POSTERS



## P1.1 - BIOFILM-FORMING PATHOGENS IN ORTHOPAEDIC IMPLANT- ASSOCIATED INFECTIONS: A CLINICAL STUDY FROM INDONESIA

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**Background & Aims:** Biofilm formation on medical implants is a key driver of antimicrobial tolerance and chronic infection. Orthopaedic implant-associated infections represent a clinically important biofilm-related disease, where structured bacterial communities embedded in extracellular polymeric substances (EPS) impair antibiotic efficacy and host clearance. This study aimed to investigate the clinical biofilm landscape of orthopaedic implant-associated infections by characterizing causative pathogens, antimicrobial resistance profiles, biofilm architecture, and the presence of selected biofilm-associated genes.

**Methods:** A cross-sectional descriptive study was conducted using bacterial isolates recovered from wound tissue cultures of patients with orthopaedic implant-associated infections at a tertiary referral hospital in Central Java, Indonesia. Bacterial identification and antimicrobial susceptibility testing were performed using the BD Phoenix™ system. Biofilm formation was quantified using a microtiter plate assay, while biofilm structure and EPS organization were visualized by scanning electron microscopy (SEM). Biofilm genetic determinants were assessed using SYBR Green-based real-time PCR targeting *bsmA* and *bsmB* genes in *Serratia marcescens* and the *icaA* gene in *Staphylococcus aureus*.

**Results:** A total of 27 bacterial isolates were obtained from 20 patients. *Serratia marcescens* was the most prevalent pathogen (18.52%), followed by *P. aeruginosa* (11.11%), *P. mirabilis* (11.11%), *S. aureus* (11.11%), *E. cloacae* (7.41%), *A. baumannii* (7.41%), *C. freundii* (7.41%), *A. veronii* (3.70%), *K. pneumoniae* (3.70%), *Acinetobacter* sp. (3.70%), *P. stuartii* (3.70%), *P. stutzeri* (3.70%), *S. haemolyticus* (3.70%), and *S. cohnii* (3.70%). The bacteria isolated in this study showed a high level of resistance to various classes of antibiotics: 57.14% of Enterobacterales isolates were ESBL producers, 100% of *A. baumannii* isolates and 33.33% of *P. aeruginosa* isolates were carbapenem-resistant. Biofilm assays confirmed robust biofilm formation, and SEM demonstrated mature, multilayered biofilms embedded within EPS matrices. All *S. marcescens* isolates carried *bsmA* and *bsmB*, and all *S. aureus* isolates were positive for *icaA*.

**Conclusions:** This study highlights the clinical relevance of biofilm structure, antimicrobial tolerance, and genetic determinants in orthopaedic implant-associated infections. The predominance of multidrug-resistant, biofilm-forming pathogens underscores the need for biofilm-informed diagnostics, prevention, and treatment strategies in implant-related infections.



## P1.2 - XPRTBIOFILM: A SIMPLE DIAGNOSTIC PLATFORM REVEALS SHEAR STRESS AS CRITICAL IN BIOFILM DEVELOPMENT

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**Background & Aims:** Biofilms are complex microbial communities that exhibit increased tolerance to antibiotics, posing significant challenges in treating chronic infections and highlighting the need for more accurate and personalized diagnostic approaches. We aimed to develop and validate the XpertBiofilm, a dynamic platform that enables physiologically relevant biofilm formation and straightforward antimicrobial susceptibility testing.

**Methods:** The XpertBiofilm device generates controlled shear stress through continuous flow, mimicking in vivo conditions encountered in chronic infections. Biofilms are formed on a removable coupon, allowing direct quantification using a standard microplate reader, without the need for complex imaging systems. XpertBiofilm validation was performed using laboratory and clinical *Pseudomonas aeruginosa* strains, as well as biofilms formed directly from cystic fibrosis sputum samples.

**Results:** Using the XpertBiofilm, a strong positive correlation between applied shear stress and biofilm biomass was established, confirming the importance of dynamic conditions for reproducible biofilm development. The platform accurately reflected known antibiotic susceptibility profiles of clinical strains. Importantly, biofilms derived directly from patient samples responded only to antibiotics to which they were clinically susceptible, supporting the clinical relevance of the system.

**Conclusions:** Overall, the XpertBiofilm provides a reliable, user-friendly, and physiologically relevant platform for studying biofilms and evaluating therapeutic strategies, offering strong potential for both basic research and personalized clinical diagnostics.



### P1.3 - DEVELOPING A MULTISPECIES APPROACH FOR ASSESSING BIOFILM FORMATION AND CONTROL IN AQUEOUS-BASED COOLING FLUIDS

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**Background & Aims:** Biofilms in industrial systems, including cooling-water environments, can exhibit high tolerance to antimicrobial agents, form complex multispecies communities, and persist under operational conditions. Routine laboratory assays used to evaluate biocide action often rely on monocultures, nutrient-rich media, or limited standardisation, reducing relevance to chemical and microbial conditions found in real industrial systems. These limitations contribute to inconsistent efficacy assessments and hinder the development of reliable antibiofilm strategies. This study aims to develop a defined multispecies approach that produces a robust, reproducible, and scalable biofilm model suitable for evaluating industrial biocides in line with Biocidal Product Regulations PT11 (Preservatives for liquid-cooling and processing systems).

**Methods:** A ten species bacterial consortium was tested, comprising *Aeromonas hydrophila*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Serratia marcescens* and *Serratia plymuthica*. Growth of each species was characterised in Model Cooling Waters (MCW) and Tryptic Soy Broth (TSB) to establish baseline behaviour under environmentally relevant and nutrient-rich conditions. Monoculture and multispecies biofilms were developed and assessed using plate-counts to quantify viable populations within the community. Fluorescence microscopy was used alongside this to determine cell viability and examine biofilm morphology. These approaches support development for reproducible, industry relevant multispecies biofilm model suitable for downstream evaluation and characterisation.

**Results:** Individual species and the consortium were able to grow in both MCW and TSB, although growth levels varied between taxa. All species apart from *Micrococcus luteus*, which was included for regulatory completeness, showed clear increases in cell density over time, enabling comparison of baseline behaviours across conditions. The defined consortium also established and grew in MCW, and selective media enabled quantification of the relative abundance of each species within the community. Fluorescence microscopy revealed differences in viability patterns and biofilm morphology between monoculture and multispecies biofilms, providing insight into structural and functional features of developing communities.

**Conclusions:** This study establishes a reproducible, standardised multispecies biofilm model that reflects industrial cooling-water conditions. By combining quantitative plate-counts with fluorescence-based visualisation, the model enables reliable assessment of biofilm structure, species persistence, and community viability, providing a robust platform for future biocide efficacy testing in line with PT11 requirements.



## P1.4 - JOINT INFECTION MULTIPLEX PCR PANEL REPURPOSING FOR SONICATION FLUID ANALYSIS IN ORTHOPEDIC IMPLANT- ASSOCIATED INFECTIONS

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**Background & Aims:** Sonication emerges as a useful diagnostic tool in implant-associated infections, enabling efficient biofilm disruption and improved microorganism isolation. However, diagnostic yield is a parameter to be improved in most laboratory settings. The aim of this small-cohort analysis was to evaluate a multiplex PCR tool, previously developed for joint infections, in the identification of etiologic agents in orthopedic implant- associated infections.

**Methods:** We conducted a retrospective analysis on a small cohort of implants sent for microbiological analysis at the 'Prof. Dr. Matei Balș' National Institute for Infectious Diseases in Bucharest, Romania. All devices underwent sonication following a standardized protocol of immersion, vortexing and sonication, followed by sonication fluid's culture on solid and enrichment media. Isolates were identified by MALDI-TOF mass spectrometry and underwent antimicrobial susceptibility testing according to EUCAST guidelines. Simultaneously, selected samples of sonication fluid were molecularly assessed, using a multiplex PCR Joint Infection Panel.

**Results:** Eighteen (n=18) samples were included in the analysis, with six definitely negative results and 12 positive cultures, including one polymicrobial infection. The multiplex PCR Joint Infection Panel retrieved negative results in all six negative cases, as well as in 5/12 positives. Amongst the non-consistent PCR-culture results, all but one infection incriminated pathogens not included in the panel (*Dermabacter hominis*, *Staphylococcus epidermidis*, *Cutibacterium acnes* and *Corynebacterium tuberculostrictum*). Additionally, a case of *Pseudomonas aeruginosa* periprosthetic infection was not detected on multiplex PCR, in which bacterial growth was modest (40 CFU/ml).

**Conclusions:** The repurposing of Joint Infection multiplex PCR panels may represent a useful adjunct in selected cases, particularly when non-cutaneous commensal pathogens are incriminated, thus enabling a timely diagnosis and individualized antimicrobial therapy. A negative rapid molecular result does not exclude infection and should be interpreted alongside conventional microbiological cultures. In contrast, a positive result obtained within one hour may significantly improve patient management by enabling earlier targeted antimicrobial therapy, optimizing clinical decisions and reducing unnecessary hospital stay.



## P1.5 - INFRARED SPECTROSCOPY FOR RAPID DIAGNOSIS OF CENTRAL LINE INFECTION

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**Background & Aims:** Blood stream infections (BSIs) result in extended hospital stays and higher mortality with central line associated blood stream infections (CLABSIs). Existing diagnostic protocols rely on correlating signs of infection with cultures from peripheral and central lines, which require significant time and cannot immediately inform clinical decision making. These methods cannot determine the microorganism phenotype at the infection site, i.e. whether a biofilm is present. Fourier transform infrared spectroscopy (FTIR) is a non- destructive, rapid technique that can reveal the exact chemical composition of a microorganism sample. The spectral data can be used to identify the infecting species and accurately indicate whether a biofilm has developed.

**Methods:** We have analysed coagulase negative staphylococci (CNS), commonly linked to BSIs, with FTIR in combination with machine learning (ML). Clinical isolates of CNS were cultured in nutrient broth media in which were suspended spectroscopically compatible slides. The slides were removed from the media, partially dehydrated and FTIR spectra and biofilm formation periodically assessed from 30 minutes to 24 hours. FTIR analysis was carried out for each sample in under 5 minutes, with a desktop spectrometer, using transmission mode in the mid-IR range (4000-400 cm<sup>-1</sup>).

**Results:** All CNS samples demonstrate reliable spectral features associated with biofilm formation. The amide I peak shift (1650 to 1635 cm<sup>-1</sup>) and merging with adjacent amide II peak, coincident with a phospholipid (1080 cm<sup>-1</sup>) spectral evolution, are noted from 30 minutes. This has been validated in two separate research facilities with multiple *Staphylococcus epidermidis* samples. ML successfully classified all planktonic samples and early-stage biofilms were distinct from later stage biofilms.

**Conclusions:** It is proposed that these spectral changes are likely due to the release of extracellular polymeric substances and may serve as an early marker for surface attachment during biofilm formation. This study underlines the practical ability of FTIR to both detect and identify bacterial species, and importantly allows characterisation of the evolving biofilm, within a clinically useful timeframe. Further work aims to enable direct continuous blood monitoring to predict CLABSIs before clinical signs of infection appear.



## P1.6 - STREPTOCOCCUS AGALACTIAE, A FREQUENT BUT NOT SO WELL- KNOWN CAUSE OF BONE AND JOINT INFECTIONS

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**Background & Aims:** Bone and Joint infections (BJI) are complex and polymorphic, associated with substantial morbidity and mortality. *Streptococcus* species represent the second most common etiological agent after *Staphylococcus*. Although *Streptococcus agalactiae* is the primary representative of streptococcal BJI, clinical and microbiological data, particularly regarding persistence mechanisms such as biofilm formation, remain markedly limited compared with staphylococcal infections. This study aimed to characterize, both clinically and microbiologically, *S. agalactiae* BJI within a large multicenter cohort and to assess strain level determinants including clonal complex distribution and biofilm forming capacity

**Methods:** We performed a retrospective observational study across six university hospitals from the CRIOGO (French national network for BJI) encompassing a 10year period. All adults with streptococcal BJI were included to identify risk factors associated with *S. agalactiae* infections. From this cohort, 176 clinical *S. agalactiae* isolates were collected. Clonal-complexes were determined by sequencing the CRISPR1 locus. Biofilm formation was quantified in 96-well plates after 48h incubation in Todd-Hewitt broth supplemented with 0.4% glucose, followed by atraumatic steam-based washing. Biofilm biomass was assessed using crystal violet staining and viable bacterial counts after sonication. Confocal laser scanning microscopy (CLSM) analyses and determination of minimal biofilm eradication concentrations (MBEC) for amoxicillin and rifampicin are ongoing.

**Results:** 1457 patients with streptococcal BJI were included. 426(29%) involved *S. agalactiae*. 950/1457(65%) were male, with median age of 67years. *S. agalactiae* was mostly implicated in hip and knee prosthetic joint infections (173/426,41%) and was associated with more chronic, less symptomatic presentations, characterized by fewer pain symptoms, more frequent sinus tracts, and lower rates of bacteremia. Peripheral arterial disease, obesity, and diabetes mellitus emerged as specific risk factors. Among the 176 isolates, CC1 (39%) and CC23 (24%) were the predominant clonal-complexes. Biofilm production was detected in 136/176 isolates (77%), with 25% classified as strong producers. No significant association was found between clonal-complex or biofilm level and clinical chronicity or treatment outcome. CLSM and MBEC results are expected by April-May 2026.

**Conclusions:** *S. agalactiae* is the leading streptococcal species involved in BJI and is strongly linked to vascular comorbidities and diabetes. The strains predominantly belonged to CC1 and CC23 and frequently produced biofilm.



## P1.7 - LOW TOXICITY OF FLAVONOID-BASED ANTIFOULING CANDIDATES ON MARINE BACTERIA

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**Background & Aims:** Marine biofouling, defined as the accumulation of organisms on submerged surfaces, results in increased fuel consumption and substantial economic losses. Current preventive strategies frequently rely on toxic coatings that may affect non-target species. Flavonoids have been proposed as environmentally sustainable antifouling alternatives. However, information regarding their effects on marine bacterial viability remains limited. This study aimed to evaluate the antibacterial profile of three flavonoid-based Nature-Inspired Antifouling Agents (NIAFs) that prevent the settlement of mussel larvae.

**Methods:** Inoculants of the marine bacteria *Alcaligenes aquatilis*, *Niallia circulans*, and *Pseudomonas toyotomiensis* were prepared in Luria Broth. Planktonic cultures were exposed to serial concentrations of the compounds ranging from 6.25 µM to 100 µM, including untreated controls. After incubation, suspensions were serially diluted in Ringer's solution and plated on solid medium. For biofilm-associated cells, biofilms were allowed to form on 9 mm-diameter round glass coverslips in the presence of 100 µM of each compound to assess inhibition of initial bacterial adhesion. After exposure, cells were detached by sonication, serially diluted, and plated. Viability was determined by colony-forming unit (CFU) enumeration.

**Results:** For the planktonic form of *A. aquatilis*, *N. circulans* and *P. toyotomiensis*, viable counts obtained after exposure to the tested flavonoids remained comparable to those of the untreated controls across the full concentration range (6.25-100 µM). Minor variations were observed, but no reproducible decreases consistent with growth inhibition were detected. Similar trends were observed for biofilm-derived populations in the presence of 100 µM of the compounds, with colony counts from biofilms formed on glass coverslips not significantly different from those in the controls.

**Conclusions:** Under the tested conditions, the evaluated flavonoids did not exhibit antibacterial activity toward the selected marine strains. These findings support the premise that such compounds may be compatible with environmentally oriented antifouling strategies by avoiding bactericidal effects while targeting other stages of the colonization succession, namely macrofouler colonization.



## P1.8 - IDENTIFYING CLINICALLY RELEVANT CANDIDA ISOLATES FOR EVALUATION OF DENDRITIC ANTIFUNGALS

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**Background & Aims:** Fungal diseases affect millions worldwide, with *Candida* species as the main cause of fungaemia. Infections are often persistent due to drug resistance and biofilm formation, which increases tolerance to antifungal agents. Dendritic systems represent a promising new approach as antifungals, demonstrating efficacy in prior studies without inducing resistance. This study characterizes clinical *Candida* isolates to select strains with high biofilm formation, antifungal resistance, and aggregation capacity, to be used in future evaluations of dendritic compounds developed in our laboratory.

**Methods:** For the characterization, five clinical isolates from each species (*Candida albicans*, *Candida auris*, *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*) were analyzed. First, the capacity to form a biofilm was assessed using the crystal violet assay. Antifungal susceptibility to commonly used commercial agents was then evaluated in order to identify the most resistant strains. Finally, the ability to aggregate was examined by microscopy.

**Results:** The results revealed a high biofilm-forming capacity in most strains, with *C. albicans* isolates showing the lowest levels of biofilm production and *C. parapsilosis* the highest. Antifungal susceptibility testing demonstrated variable resistance patterns across all species. In contrast, aggregation assays showed that most strains did not exhibit significant aggregation.

**Conclusions:** In conclusion, this study allowed the identification of clinically relevant *Candida* isolates with high biofilm formation capacity and diverse antifungal resistance profiles. These strains represent a suitable basis for the future evaluation of dendritic compounds as potential antifungal agents.



## P1.9 - DIAGNOSTIC YIELD OF CULTURE OF SYNOVIAL FLUID, PERIPROSTHETIC TISSUE AND SONICATE FLUID IN PATIENTS UNDERGOING DEBRIDEMENT, ANTIBIOTICS AND IMPLANT RETENTION FOR PERIPROSTHETIC JOINT INFECTION

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1 - Mayo Clinic

**Background & Aims:** Periprosthetic joint infection (PJI) is a devastating biofilm-associated disease; definition of microbiology guides antimicrobial treatment. Preoperatively, synovial fluid may be collected, and intraoperatively, periprosthetic tissues and resected implant components may be collected, for culture, but sensitivities are imperfect. In PJI managed with debridement, antibiotics and implant retention (DAIR), removable components may be subjected to sonication, but the entire implant is not available for testing; culture-based yield of this process is incompletely defined. The aim of this study was to evaluate the yield of synovial fluid, periprosthetic tissue and sonicate fluid culture in patients undergoing DAIR for PJI management.

**Methods:** Synovial fluid, periprosthetic tissue and sonicate fluid cultures from patients with hip or knee PJI who underwent DAIR, between January 2012 and December 2022, were studied. PJI diagnosis was based on IDSA guidelines. Tissue cultures were considered positive if at least 2 grew the same microorganism or 1 grew a virulent microorganism (e.g., *Staphylococcus aureus*). For sonication, 400 mL Ringer's solution was added to components in solid containers, followed by vortexing and sonication, centrifugation, and plating to aerobic and anaerobic solid media; culture results were reported semiquantitatively with growth above 19 CFU/10 mL sonicate fluid or any growth of a virulent microorganism considered positive.

**Results:** 62 PJIs were evaluated, 77% and 33% from knees and hips, respectively. Median age was 67 years; 52% were male. Positivity of synovial fluid, periprosthetic tissue and sonicate fluid culture was 75%, 68% and 65%, respectively. The combination of periprosthetic tissue and synovial fluid culture and the combination of synovial fluid and sonicate fluid culture had 77% sensitivity; the combination of periprosthetic tissue and sonicate fluid culture had 71% sensitivity; and the combination of 3 specimen-types had 79% sensitivity. Isolated microorganisms were concordant in all except 7 cases; in discordant cases, the same organism was detected in 2 or 3 specimen-types, with 1 or more additional organisms detected in 1 specimen-type.

**Conclusions:** The culture-based yield of sonicate fluid was not superior to synovial fluid or periprosthetic tissue cultures in subjects undergoing DAIR. The highest culture positivity rate was achieved with culture of all three specimen-types.



## **P1.10 - MINING NOVEL ANTIMICROBIAL PEPTIDES FROM HYDROCARBON-CONTAMINATED ENVIRONMENTS: TARGETING ESCAPE PATHOGENS AND BIOFILMS.**

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1 - QUEEN'S UNIVERSITY BELFAST

**Background & Aims:** Antimicrobial resistance (AMR) is widely recognised as one of the most serious global health challenges, highlighting the need for new antimicrobial agents. This study investigates hydrocarbon-contaminated soils in Nigeria as sources of antimicrobial peptides (AMPs), utilising microbial adaptations to oil pollution to fight AMR. Pollution alters microbial communities, enhancing bioactive compound production, including antimicrobial peptides.

**Methods:** Fifteen soil/sediment samples from five Nigerian hydrocarbon-impacted sites were analysed through a metagenomic approach. These involved DNA extraction, Illumina sequencing, contig assembly, ORF prediction, and AMP mining, used for antimicrobial testing and biofilms.

**Results:** Identifying 73 (AMPA) and 95 (Macrel) AMPs, refined to 12 and 28 candidates via APD3 criteria. Additionally, 38 Metagenome-Assembled Genomes (MAGs) were generated with >90% completeness and <10% contamination, with an AMPA of 10,543 and Macrel of 111,064 AMPs streamlined to 358 and 3, respectively, for synthesis. Ten candidate AMPs were synthesised; three exhibited activity. ZB8 displayed potent MICs of 16 µg/mL against MRSA 33592 and *S. aureus* ATCC 43300, 32 µg/mL against *E. faecium* DSM 25390 and *E. coli* ATCC 10488, and 64–128 µg/mL against *K. pneumoniae* NCTC 204 and *A. baumannii* NCTC 13304, with MBCs of 128–256 µg/mL. Biofilm assays confirmed MBIC/MBEC endpoints.

**Conclusions:** These results suggest that oil-contaminated Nigerian sites demonstrate a rich diversity of AMPs in oil-impacted soils, advancing bioprospecting for AMR solutions. Future efforts include expanded synthesis, optimisation and toxicity profiling and will confirm therapeutic potential.



## **P1.11 - PRELIMINARY STUDY ON THE APPLICATION OF HYPERSPECTRAL IMAGING IN THE ASSESSMENT OF BIOFILM FORMED BY COAGULASE- POSITIVE STAPHYLOCOCCI (S. AUREUS) AND COAGULASE- NEGATIVE STAPHYLOCOCCI (CONS) COLLECTED FROM CARDIAC IMPLANTABLE ELECTRONIC DEVICE-RELATED INFECTIONS**

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**Background & Aims:** Cardiac implantable electronic device (CIED) infections increase patient morbidity, mortality, and costs of long-term antibiotic therapy. The most common etiological factors are biofilm-forming, often multidrug-resistant Gram-positive cocci. The study aimed to evaluate the feasibility of using hyperspectral imaging to assess bacterial biofilm on the CIED surface.

**Methods:** Swabs were collected from three CIED generator pockets and endocardial leads. Six *S. aureus* and six CoNS biofilms were cultured in 6-well plates for HSI analysis. After removing growth fluid, plates were imaged with a SPECIM IQ camera (420–980 nm range, 5 nm spectral resolution, 512×512 pixels spatial resolution, 17.58×17.58 μm). Eight points per plate (including an empty control plate) were marked, generating 48 SNV-normalized spectral curves in MATLAB. Reflectance of the empty plate was subtracted. A total of 24 CoNS and 24 *S. aureus* curves were obtained at 24 and 48 h. K-means clustering was applied to normalized spectra using the full range and the key range 617–788 nm. Clustering performance for *S. aureus* was assessed using Recall (R), Precision (P), F1-score (F1S), and Classification Accuracy (CA). The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice, Poland (BNW/NWN/0052/KB1/83/23).

**Results:** After 24 hours in the full spectral range, the following values were obtained: R= 1, P=0.6, F1 S=0.75, and CA=66.7%; while for the spectral range 617–788 nm, these classification metrics were: R=1, P=0.68, F1 S=0.81, and CA=77.1%. In turn, after 48 h, the model achieved a classification accuracy of 87.5% across both the full spectral range and the 617–788 nm range. The remaining classification metrics also showed the same values of R=1, P=0.8, and F1S=0.89 for the entire spectral range and for the 617–788 nm spectral range.

**Conclusions:** HSI may be a promising method for distinguishing Staphylococci biofilms, with a recall value of 1 after 24 and 48 hours of incubation. However, its precision and classification accuracy at this stage encourage further analysis of research methodology. The National Recovery and Resilience Plan supported the study as part of a project entitled "Modern diagnostics and treatment regimens for infections of implantable cardiac devices with drug-resistant bacterial strains" (Grant No 2024/ABM/03/KPO/KPOD.07.07-IW.07-0004/24-00).



## P2.1 - IS 2D AND 3D-OCT IMAGING INFORMATION COMPARABLE FOR PSEUDOMONAS FLUORESCENS BIOFILMS GROWN IN A CDC REACTOR?

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**Background & Aims:** Biofilms are structured microbial communities attached to surfaces. Since biofilms are 3-dimensional heterogeneous structures, there is a need for imaging techniques which allow their proper visualization, qualitative description and quantification. One of those techniques is Optical Coherence Tomography (OCT), which can be used to visualize the mesoscale structure of biofilms. Although both 2D and 3D-OCT can be used to study the biofilm mesoscale structure, 3D-OCT is a potential improvement over 2D-OCT, as each 3D image combines a set of 509 adjacent 2D images. Therefore, the main aim of the present work was to compare the qualitative and quantitative information obtained with 2D and 3D-OCT images. In addition, this work aimed at understanding if a CDC (Center for Disease Control) biofilm reactor can generate biofilms with consistent and homogeneous properties regarding the mesoscale structure.

**Methods:** *Pseudomonas fluorescens* biofilms were grown in a CDC (Center for Disease Control) biofilm reactor under a stirring velocity of 125 RPM or 225 RPM and were sampled after 7 days for OCT imaging. The OCT images were subsequently processed to quantify biofilm structural parameters such as thickness, porosity, compaction and roughness.

**Results:** Average biofilm thickness values obtained through 3D-OCT imaging were higher than those obtained through 2D-OCT ( $108 \pm 38 \mu\text{m}$  vs.  $85 \pm 25 \mu\text{m}$ ), for a stirring speed of 125 RPM. Additionally, biofilms imaged through 3D-OCT were found to be more compact under both rotating speeds of 125 RPM and 225 RPM. Besides this, the biofilms, which were grown in a CDC biofilm reactor, presented identical values of thickness, compaction, roughness or porosity regardless of their position within the reactor.

**Conclusions:** The results suggest that using 2D-OCT imaging alone might result in a misleading analysis of the mesoscale structure of the biofilm. Thus, 3D-OCT is necessary for a more accurate characterization and visualization of the heterogeneous structure of biofilms. Moreover, the mesoscale structure of the biofilms grown in the CDC biofilm reactor was consistent across the whole vessel.



## P2.2 - MECHANOSENSITIVE REGULATION OF BIOFILM DYNAMICS BY QSEBC IN KLEBSIELLA PNEUMONIAE

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**Background & Aims:** *Klebsiella pneumoniae* is an enterobacterial pathogen responsible for nosocomial infections and belongs to the ESKAPEE group, whose multidrug resistance to antibiotics represents a major public health concern. In addition, its ability to form biofilms contributes to treatment failure and persistence of *K. pneumoniae* infections. Biofilm dispersion, a process by which bacteria escape from the biofilm structure to colonize new sites, plays a key role in host colonization and infection dissemination. However, the mechanisms regulating this process remain poorly characterized in *K. pneumoniae*. The aim of this work is to investigate the biofilm dynamics of *K. pneumoniae* and to identify the molecular mechanisms involved in the transition between biofilm-associated and dispersed states.

**Methods:** A random mutagenesis-based mutant library screening identified the QseBC two-component regulatory system (TCS) as a potential determinant of biofilm dispersion. The biofilm phenotype of the  $\Delta qseB$  and  $\Delta qseC$  mutants was first quantified under static conditions (multiwell plate) in multiple conditions (pH, carbon source, anaerobic condition...). Then, biofilm formation kinetics was monitored under mechanical stress using a microfluidic device and observed in real time using epifluorescence microscopy. Finally, the transcriptomic profiles of each mutant were analyzed on biofilm samples by whole-transcriptome sequencing (RNA-seq) to identify genes regulated by QseBC in the biofilm.

**Results:** Under static conditions or low-shear stress, no differences in biofilm formation were observed between the mutants and the wild-type strain in each condition tested. In contrast, under mechanical shear stress, both mutants developed biofilm that were more robust and more resistant to erosion than the wild-type strain. Global transcriptomic analysis further indicates that QseBC TCS could be involved in the regulation of quorum-sensing and biofilm matrix composition. Taken together, these results suggest that QseBC is activated in response to mechanical stress and modulated biofilm dynamic by regulating bacterial cohesion and/or matrix composition.

**Conclusions:** Ongoing work aims to validate mechanical shear stress as an activation signal of QseBC, to demonstrate how this TCS controls quorum sensing and biofilm matrix composition, and to elucidate how these regulations, in turn, govern biofilm dynamics through QseBC in response to shear stress.



## P2.3 - CONTACT-KILLING EFFICACY OF CHITOSAN-BASED HYDROGELS AGAINST STAPHYLOCOCCI FOR WOUND DRESSING APPLICATION

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**Background & Aims:** Diabetic foot ulcers (DFUs) are frequently complicated by *Staphylococcus epidermidis*, whom antimicrobial resistances drive to chronic infections and therapeutic failures. Innovative wound dressing strategies are urgently needed to overcome these challenges. Here, we report the development of multifunctional chitosan (CS)-based hydrogel polymerized with acrylamide (PAM) and incorporating cellulose (Cell) to enhance mechanical stability and antimicrobial efficacy against staphylococcal pathogens associated with DFUs.

**Methods:** Two chitosan molecular weights were used, and the corresponding hydrogels were designated as chitosan with low (CS(LMW)/PAM) and high (CS(HMW)/PAM) molecular weight. The hydrogels were prepared by free radical polymerization of acrylamide using ammonium persulfate as a thermal initiator and N, N'-methylenebisacrylamide as a chemical crosslinker. For composite hydrogels, microcrystalline cellulose was gradually incorporated into the CS/PAM precursor solution. Polymerization was carried out at 65°C for 2h, followed by washing with deionized water and storage at 4°C. Antimicrobial activity was assessed against *Staphylococcus epidermidis* EDCC 5130 and *Staphylococcus aureus* JAR060131. Bacterial suspensions in mid-logarithmic growth phase were spread onto LB agar plates and samples were placed on the surface. Following 18 h incubation at 37°C, inhibition zones were quantified using ImageJ software. Contact-killing properties were evaluated using the Japanese Industrial Standard (JIS) (ISO 22196). After 24h incubation at 37°C, viable bacteria were quantified by CFU enumeration.

**Results:** Agar diffusion assays revealed inhibition zones against both species, with *S. epidermidis* more susceptible. JIS assays performed with *S. epidermidis* demonstrated antibacterial activity for both chitosan-based hydrogels with added cellulose. No significant reductions were observed against *S. aureus* under contact conditions and agar diffusion assays. From those methods, we noticed that the addition of cellulose into chitosan-based hydrogel significantly reduced bacterial attachment particularly for CS(LMW)/PAM-based formulations.

**Conclusions:** Chitosan-based hydrogels formulated with cellulose demonstrated promising contact-killing activity against *S. epidermidis*, a major pathogen associated with DFUs. The addition of cellulose did not broaden the antimicrobial spectrum. Further optimizations may enhance efficacy against more resistant pathogens such as *S. aureus*, supporting the development of advanced preventive wound care strategies.



## P2.4 - QUANTIFYING THE ROLE OF NUTRIENT ADSORPTION IN EARLY BIOFILM EXPANSION

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**Background & Aims:** Understanding why early-stage biofilms often exhibit different biomass accumulation rates compared to planktonic populations remains a fundamental challenge in microbial ecology. Traditional measurements frequently fail to distinguish whether this enhancement arises from intrinsic surface-sensing physiology or from localized nutrient enrichment at the solid-liquid interface. Here, we employ single-cell 3D kinetic analysis across diverse taxa to mechanistically isolate the drivers of initial surface colonization. By comparing planktonic and surface-associated populations in adsorptive and non-adsorptive environments, we examine whether rapid early biofilm expansion is driven by surface-mediated nutrient organization rather than physiological reprogramming.

**Methods:** We developed a high-resolution imaging workflow using *Pseudomonas fluorescens* WCS365 and extended the approach to include the Gram-positive model *Bacillus subtilis* NCTC 3610. Cells were grown on untreated glass Ibidi  $\mu$ -Slide 8-well chambered coverslips in defined M9-glucose minimal medium with or without casamino acids and imaged by confocal laser scanning microscopy (CLSM). Time-resolved single-cell growth trajectories were extracted using machine-learning-based segmentation with StarDist and BiofilmQ. To enable direct comparisons, planktonic specific growth rates ( $\mu$ ) were independently determined in aerated shake-flask assays to minimize wall-associated growth artifacts.

**Results:** In complex medium, surface-associated cells reached a  $\mu$  of  $1.04 \text{ h}^{-1}$ , significantly higher than planktonic counterparts. This substantial enhancement suggests that early biofilm growth may be influenced by surface-associated nutrient accumulation rather than intrinsic physiological activation. To directly assess causality, ongoing experiments compare growth dynamics in M9-glucose minimal medium, which lacks strongly adsorptive components, and in casamino acid-supplemented conditions. In parallel, surface PEGylation of glass coverslips is being implemented to inhibit conditioning film formation and evaluate its impact on surface-associated growth rates.

**Conclusions:** These findings support our hypothesis that the early biofilm growth advantage is an emergent physicochemical phenomenon driven by microscale nutrient adsorption at the solid-liquid interface, rather than attachment-induced physiological activation. If surface-mediated enrichment is the primary driver, the kinetic divergence between planktonic and surface-associated cells will be abolished in non-adsorptive media or on PEGylated non-fouling surfaces. By integrating single-cell kinetics with controlled surface and media conditions, we establish a quantitative framework to disentangle physicochemical and biological drivers of early biofilm development, informing biofilm control strategies in clinical and industrial settings.



## P2.5 - LINKING BIOFILM DISPERSION TO MEMBRANE VESICLE PRODUCTION IN *PSEUDOMONAS AERUGINOSA*

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**Background & Aims:** A broad range of bacterial species release membrane vesicles (MVs), nanosized spherical structures (20-400 nm), from their membranes. MVs composed of bacterial substances are enriched in bacterial biofilms and have multiple functions, including toxin delivery, inter kingdom communication, and modulation of host immune responses. Since large amounts of MVs can be trapped in high-viscosity biofilms, how they contribute to delivery to distant cells remains a mystery. To resolve this apparent contradiction, we focused on spontaneous biofilm dispersion. Here, we show that biofilm dispersion factors, Dspl and PA0744, are involved in MV production in *Pseudomonas aeruginosa* biofilms.

**Methods:** For biofilm conditions, bacterial cultures were grown statically in Petri dishes at 37°C. MV production was evaluated by measuring phospholipid concentration. The cell-free supernatant was ultracentrifuged at 150,000 × *g* at 4°C. The MV pellets were resuspended in HEPES-NaCl buffer and incubated with 5 µg/mL FM4-64 at 37°C for 30 min in the dark. The fluorescence intensity was measured at 506/750 nm (excitation/emission wavelength) and the fluorescence intensity was presented as the phospholipid amount in vesicles normalized to the cell density.

**Results:** Deletion of *dspl*, which encodes an enzyme for the biosynthesis of a biofilm dispersal signal *cis*-2-decenoic acid (CDA), or deletion of the adjacent gene *PA0744* significantly repressed MV production under static biofilm conditions. In their respective mutants, no CDA synthesis was detected, and the dispersion of biofilm was repressed. Interestingly, the addition of exogenous CDA did not restore MV production in the *dspl* mutant, suggesting that the promotion of MV formation by these factors in biofilms is a mechanism independent of the CDA signaling pathway. In addition, the fatty acid and protein composition of mutant derived MVs differed from that of wild type, suggesting that Dspl and PA0744 are involved in the regulation of enhanced MV production and the role of MVs in *P. aeruginosa* biofilms.

**Conclusions:** Our study demonstrated that biofilm dispersion factors regulate MV production in *P. aeruginosa* biofilms. This study provides new insight into how bacteria coordinate MV production with the biofilm lifecycle.



## P2.6 - SEDIMENTATION CONTRIBUTED TO HIGHER BIOFILM THICKNESS AND BIOFILM STRUCTURE HETEROGENEITY

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**Background & Aims:** Biofilm structure plays a crucial role in protecting microorganisms and resisting detachment under external stresses. Hydrodynamic conditions influence biofilm microbiome composition and structural characteristics. Particularly, water stagnation (no flow), a common issue in engineered water systems, is associated with water quality deterioration, increased microbial growth, and microbial population shifts. For example, stagnation fosters *Legionella* colonization of existing biofilms, raising concerns for Legionnaire's Disease prevention. In stagnant pipelines, biofilms form in varying orientations, and this study explores whether they impact biofilm structure differently under stagnant conditions.

**Methods:** *Pseudomonas fluorescens* biofilms were developed under flow conditions ( $Re=4440$ ) for 11 days in a laboratory flow cell equipped with coupons and Surface Sensor Technology for real-time biofilm monitoring. The system was then stopped for 48 h with the flow cells placed in the upright position, horizontally with the coupons on top or horizontally with the coupons on the bottom. Flushing was performed after under the same flow conditions as the first 11 days ( $Re=4440$ ) for 15 min. Coupons were periodically sampled; biofilm structure was analysed through Optical Coherence Tomography and culturability was assessed to evaluate optimal biofilm formation.

**Results:** Preliminary findings suggest that stagnation effects vary with biofilm positioning. When biofilms remain in a vertical position, thickness decreases, whereas coupons on the bottom result in increased thickness. Those differences may be due to gravity effect on biofilms or suspended solids in the bulk phase. Flushing does not seem to impact biofilm thickness in any flow cell orientation.

**Conclusions:** Stagnation may impact biofilm detachment patterns, leading to heterogeneous structural adaptations depending on flow history. Further research is needed to clarify the mechanisms and the implications in biofilm persistence. Understanding how stagnation influences biofilm behaviour is important to develop effective water management strategies and improve biofilm control.



## P2.7 - PLANT-INSPIRED MICROTOPOGRAPHIES TO REDUCE E. COLI BINDING ON FOOD CONTACT SURFACES

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**Background & Aims:** Bacterial contamination of food contact surfaces remains a major challenge for the food industry due to the ability of pathogens to adhere and persist on this environment. Conventional sanitation methods may be insufficient when bacteria become resistant to cleaning and disinfection. Biomimetic surfaces inspired by naturally antifouling plant microtopographies are a promising strategy to reduce bacterial binding without relying on chemicals/antimicrobials. This study aimed to evaluate the potential of vegetable-inspired silicone surfaces to reduce *Escherichia coli* binding, both in the presence and absence of a casein-conditioning film, assessing whether antifouling activity is preserved under realistic food-processing conditions.

**Methods:** The topography of four vegetable leaves, Tenderheart (TH), Cauliflower (CF), White Cabbage (WC), and Leek (L), was replicated using a moulding technique. Bare and casein-conditioned surfaces were analysed regarding their morphology and roughness by optical profilometry and scanning electron microscopy, respectively, while their wettability and chemistry were assessed by water contact angle measurements and Raman spectroscopy, respectively. The antifouling activity of the biomimetic surfaces against *E. coli* was evaluated by a 10-second spray-and-wash attachment assay, and a 1-hour static incubation retention assay.

**Results:** All biomimetic surfaces showed higher roughness and water contact angles than the flat control, with WC being the roughest and L the least wettable. Bare silicone biomimetic surfaces significantly reduced *E. coli* attachment and retention compared to a flat control, demonstrating the strong influence of the replicated microstructures. Following attachment assays, the number of *E. coli* attached was reduced by 88% on the CF and 78% on TH, WC, and L, while retention assays showed reductions between 58-82% for L and WC, respectively. Conditioning with casein increased surface wettability and partially masked surface microtopography. However, conditioned biomimetic silicone surfaces maintained their antifouling activity, achieving reductions in bacterial attachment up to 90% for L, and decreases in retention of up to 69% for WC. Overall, surfaces with increased roughness and low wettability displayed the strongest inhibition of *E. coli* binding, even in the presence of casein.

**Conclusions:** These findings support the potential of vegetable-inspired silicone surfaces in providing an effective strategy to minimise bacterial contamination in food contact materials and improve food safety.



## P2.8 - SELECTIVE CAPTURE OF MICROPLASTICS BY ENVIRONMENTAL BIOFILM-FORMING BACTERIA ISOLATED FROM PLASTIC DEBRIS

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**Background & Aims:** Environmental bacteria and biofilms influence the fate of microplastics (MPs) in aquatic systems, yet the mechanisms and strain-level variability underpinning MP capture remain unclear. We aimed to quantify differences in MP aggregation capacity across bacterial isolates from plastic debris and surrounding water, and to identify factors contributing to observed boundaries in MP removal from suspension.

**Methods:** A collection of environmental bacterial strains was isolated from plastic residues and adjacent water in a river system. Polystyrene MPs (0.5  $\mu\text{m}$ ) were incubated with each isolate under both dynamic (gentle agitation mimicking natural particle movement) and static conditions. MPs remaining in suspension were quantified after 24 h to assess removal efficiency. Diffusion-limited encounter rates between MPs and cells were estimated using classical Smoluchowski theory to contextualize observed kinetics, and the efficiency of aggregation was characterized with an effective collision efficiency parameter ( $\alpha$ ).

**Results:** Strains isolated from plastic debris were significantly more likely to promote MP aggregation than those from the surrounding water column. The most efficient isolates consistently reduced MP concentrations from an initial  $10^8$  MPs  $\text{mL}^{-1}$  to a lower plateau near  $10^6$  MPs  $\text{mL}^{-1}$ , establishing a reproducible  $\sim 2$ -log removal limit. Aggregation dynamics were influenced by incubation conditions; rolling conditions enhanced removal for many strains, whereas static conditions reduced efficiency for some isolates. Among the best performers, *Pseudomonas parakoreensis* A16 showed robust MP capture across conditions, followed by *Acinetobacter johnsonii* A20 and *Deinococcus aquaticus* P5. Diffusion theory indicated high potential encounter rates, but an effective collision efficiency ( $\alpha \sim 10^{-2}$ ) suggests that only a fraction of encounters yield sedimenting aggregates.

**Conclusions:** MP capture is a strain-dependent trait enriched among plastic-associated bacteria, and is modulated by physical conditions that alter encounter dynamics. The consistent two-log removal ceiling likely reflects a combination of limited adhesion efficiency and sedimentation of formed aggregates. These findings emphasize the role of microbial diversity and biofilm-associated traits in shaping microplastic fate in freshwater environments.



## P2.9 - FROM ANTIOXIDANT TO ANTIBIOFILM AGENT: ASCORBIC ACID OUTPERFORMS CITRIC ACID AGAINST EARLY AND MATURE BIOFILMS

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**Background & Aims:** Biofilms are structured bacterial communities that persist on surfaces, showing resistance to conventional disinfectants. Their presence in healthcare and domestic settings poses contamination risks. Citric and ascorbic acids are safe and inexpensive compounds with antimicrobial potential. However, their ability to disrupt preformed biofilms, particularly mature biofilms, is poorly characterized. This study aimed to evaluate and compare the antibiofilm activity of citric and ascorbic acids against *Escherichia coli* and *Staphylococcus aureus* biofilms at early (24 h) and mature (48 h) stages.

**Methods:** Minimum inhibitory concentrations (MICs) against planktonic *E. coli* ATCC 8739 and *S. aureus* ATCC 6538 were determined by broth microdilution in Mueller–Hinton broth from 50 mg/mL stock solutions. MICs for citric acid were 1.56 mg/mL for *S. aureus* and 3.125 mg/mL for *E. coli*, and for ascorbic acid, 6.25 mg/mL for both species. Biofilms were formed in 96-well polystyrene plates at 22°C for 24 or 48 h in Luria–Bertani broth. After washing, preformed biofilms were exposed for 4 h to acids at MICs. Biofilms were washed, sonicated (5 min), scraped, serially diluted, and viable cells enumerated (CFU/mL). Antibiofilm activity was expressed as log<sub>10</sub> reduction relative to untreated controls. Experiments performed in triplicate.

**Results:** At 24 h, citric acid reduced *E. coli* biofilm by 0.60 log at 1.56 mg/mL and 1.12 log (92.5%) at 3.125 mg/mL. Ascorbic acid achieved 0.97 log (89.2%). For *S. aureus* 24 h biofilms, citric acid caused 0.45 log and 0.54 log reductions at 1.56 and 3.125 mg/mL, while ascorbic acid achieved 1.21 log reduction (93.8%). At 48 h, citric acid activity declined in biofilms and promoted growth (+0.26 log at 1.56 mg/mL; +0.38 log at 3.125 mg/mL). Ascorbic acid at 48 h, reduced by 0.91 log (87.7%) and by 0.44 log (63.8%).

**Conclusions:** Ascorbic acid demonstrated consistent antibiofilm activity across species and biofilm maturation stages. Citric acid showed concentration-dependent activity in early biofilms but lost efficacy in mature structures and promoted *S. aureus* growth. Under these conditions, ascorbic acid is the more reliable antibiofilm agent, supporting its potential as an effective strategy for surface biofilm control.



## P2.10 - SHEAR-DRIVEN BIOFILM DETACHMENT: TIME-RESOLVED ANALYSIS OF SIZE SPECTRA IN STAPHYLOCOCCUS EPIDERMIDIS BIOFILM

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**Background & Aims:** Biofilm detachment represents a critical yet poorly understood stage in the biofilm life cycle. Detachment may occur through erosion or sloughing, processes influenced by both biofilm architecture and hydrodynamic shear. Quantitative analyses have demonstrated that detachment generates highly skewed size distributions comprising single cells and aggregates, with infrequent large events contributing disproportionately to total biomass release. Although hydrodynamic stress influences detachment patterns, most quantitative analyses characterize size distributions from collected effluent samples, providing limited temporal resolution of shear- dependent size evolution. The present study investigates how controlled hydrodynamic regimes regulate the size distributions and release dynamics of detached entities from *Staphylococcus epidermidis* biofilms under continuous flow conditions.

**Methods:** Biofilms were cultivated in Ibidi flow-cell systems under three distinct flow rates representing distinct shear conditions. A dual-chamber microfluidic setup was developed to enable real-time capture and tracking of detached cells and aggregates. Effluent from the primary biofilm chamber was continuously transferred into a second flow chamber for live imaging using confocal laser scanning microscopy. Aggregate size distribution and detachment kinetics were quantified using automated image analysis workflows. In parallel, primary biofilms grown under each flow rate were characterized using EPS-specific fluorescent staining and confocal microscopy to evaluate shear-dependent differences in matrix composition and structural architecture at the detachment interface.

**Results:** Detachment generated heterogeneous populations comprising single cells and multicellular aggregates across all shear conditions. Size distributions were highly skewed, with distinct differences in detachment frequency observed across the imposed flow rates. Temporal analysis revealed dynamic shifts in size spectra during biofilm maturation under continuous flow. Characterization of primary biofilms grown under each flow condition revealed shear-dependent differences in structural organization and EPS distribution. These variations in matrix architecture corresponded with differences in the size characteristics of detached entities released from the biofilm.

**Conclusions:** Hydrodynamic conditions regulate both the frequency and size distributions of detached entities released from *S. epidermidis* biofilms under continuous flow. Time-resolved imaging combined with matrix characterization provides an integrated framework for examining shear-dependent detachment phenotypes. This work advances quantitative understanding of biofilm detachment under continuous flow and supports improved mechanistic interpretation of erosion and sloughing-associated release processes.



## P2.11 - DOMIPHEN BROMIDE-FUNCTIONALIZED 3D-PRINTED PCL SURFACES PREVENT BIOFILM FORMATION BY CLINICAL BILIARY ISOLATES

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**Background & Aims:** Biofilm formation on indwelling medical devices is a major cause of healthcare-associated infections and device failure. In plastic biliary stents, microbial biofilm accumulation promotes progressive luminal occlusion and frequently necessitates premature device replacement. Preventing microbial adhesion on device surfaces is therefore a key clinical priority. Domiphen bromide (DB), a quaternary ammonium compound with broad-spectrum antibacterial and antifungal activity, represents a potential candidate for device functionalization; however, its antibiofilm activity in device-associated settings remains poorly defined. This study evaluated the antimicrobial and antibiofilm activity of DB against clinical isolates recovered from explanted biliary stents and explored its incorporation into a 3D-printable polycaprolactone (PCL) matrix as a prototype antibiofilm material.

**Methods:** Antimicrobial activity against five biliary stent isolates (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Candida albicans*) was determined by broth microdilution (MIC and MCC). Antibiofilm activity was assessed by XTT assay. Unmodified or DB-functionalized PCL discs were fabricated by 3D printing. Anti-biofilm efficacy after 48 h of biofilm formation was evaluated by quantitative CFU enumeration and scanning electron microscopy (SEM). To assess durability after compound release, functionalized discs were tested either immediately or following 7-day pre-incubation in culture medium prior to microbial challenge. *In vivo* toxicity was evaluated using the *Galleria mellonella* model, and *in vitro* cytotoxicity was assessed on murine 3T3 fibroblasts.

**Results:** DB was active against all isolates. MIC/MCC values were 4 µg/mL for gram-negative and 1 µg/mL for gram-positive, while *C. albicans* showed MIC/MCC of 2 µg/mL. DB reduced biofilm metabolic activity in a dose-dependent manner. DB-functionalized PCL discs completely prevented biofilm formation by *C. albicans*, *K. pneumoniae*, and *E. faecalis*, with undetectable CFU/mL and absence of adherent cells by SEM, whereas blank discs showed extensive colonization. Anti-biofilm activity was retained after 7-day pre-incubation. No mortality was observed in *Galleria mellonella* larvae exposed to DB (up to 40 µg/mL, 72 h). Eluates from DB-functionalized discs didn't induce cytotoxicity in 3T3 fibroblasts up to 72 h.

**Conclusions:** DB-functionalized PCL surfaces demonstrated anti-biofilm activity against clinically relevant biliary pathogens. Ongoing work includes fabrication of DB-functionalized 3D-printed biliary stents and their evaluation under dynamic continuous-flow conditions to better reproduce the biliary environment.



## P2.12 - INTERPLAY BETWEEN COAGGREGATION AND EXTRACELLULAR METABOLITES SHAPES BIOFILM FORMATION

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**Background & Aims:** Multispecies biofilms are central to chronic infections and device-associated contaminations, where interbacterial interactions enhance persistence and antimicrobial tolerance [1,2]. Although bacterial coaggregation is recognized as a structural driver of biofilm assembly in oral ecosystems, its role in clinically relevant non-oral systems remains poorly characterized [3]. This study aimed to identify novel coaggregating clinical strains and disentangle the relative contributions of direct cell-cell interaction and extracellular metabolites in shaping multispecies biofilm development.

**Methods:** Pairwise aggregation assays were performed among several bacterial strains. Biofilm formation was evaluated in single- and dual-species systems, as well as in biofilms supplemented with cell-free supernatants (CFS). Biofilm thickness and roughness were assessed by optical coherence tomography (OCT), culturability by CFU enumeration, and extracellular polymeric substance (EPS) composition was quantified. NMR-based metabolomics was used to characterize extracellular metabolic profiles.

**Results:** *Escherichia coli* ATCC 25922 was identified for the first time as a coaggregating strain, interacting with *Staphylococcus saprophyticus*. Although coaggregation influenced biofilm structural heterogeneity, the most significant effects were observed in the presence of extracellular metabolites. CFS supplementation significantly increased biofilm thickness, culturability, and EPS polysaccharide and lipid content, often exceeding the biomass observed in dual-species biofilms. Metabolomic analysis revealed strain-specific metabolic signatures, including exclusive citrate secretion by the coaggregating strain, suggesting environmental modulation as a potential driver of interspecies interactions.

**Conclusions:** These findings demonstrate that extracellular metabolic activity may exert a stronger influence on biofilm formation than physical coaggregation alone. In clinically relevant contexts, metabolic cross-talk may enhance biofilm resilience and persistence independently of direct adhesion mechanisms. Importantly, the identification of *E. coli* ATCC 25922 as a coaggregating strain expands the diversity of recognized bridging bacteria beyond the oral microbiome and provides an accessible, well-characterized model for systematically studying interspecies interactions in clinically relevant biofilms.



## P2.13 - GLUCOSE-GENTAMICIN INTERPLAY GOVERNS SURFACE- ASSOCIATED MOTILITY AND BIOFILM DYNAMICS IN PSEUDOMONAS AERUGINOSA ISOLATE HRW.1-S3

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**Background & Aims:** Surface-associated behaviours in *Pseudomonas aeruginosa* involve a tightly regulated transition between swarming motility and biofilm formation. However, how environmental cues coordinate this transition at the surface level remains poorly understood. This study aimed to elucidate how glucose, a ubiquitous carbon source and sub-inhibitory gentamicin modulate the swarming-to-biofilm transition in *P. aeruginosa* HRW.1-S3.

**Methods:** Swarming assays and biofilm quantification were performed under varying glucose and gentamicin concentrations. Extracellular polymeric substances (EPS) were biochemically quantified. Surface architecture and cellular morphology were analysed using scanning electron- and confocal microscopy. Expression of motility, quorum sensing, and biofilm-associated genes was assessed using RT-qPCR. A *lasI* deletion mutant was constructed to understand the role of quorum sensing in regulating these responses.

**Results:** Glucose exhibited a concentration-dependent regulatory effect on surface behaviour, with lower concentrations (0.25%) supporting swarming, whereas higher concentrations (0.5%) inhibited motility and promoted biofilm formation. Biofilm biomass and EPS production increased proportionally with glucose, indicating a shift towards a sessile lifestyle. Interestingly, the sub-inhibitory doses of gentamicin further modulated these behaviours in a dose-dependent manner: 0.75 µg/mL enhanced swarming across conditions, whereas 1.5 µg/mL suppressed motility and reinforced biofilm formation, suggesting a stress- adaptive transition. Notably, the combined presence of elevated glucose and gentamicin resulted in maximal biofilm development, reflecting a synergistic interplay that drives surface colonization. Microscopy revealed distinct surface reorganisation, with swarming tendrils transitioning into dense and adherent biofilm structures under high glucose and antibiotic stress. Gene expression analysis showed up-regulation of *fliA* and *rhIB* genes during active surface motility, while *pqsA* was enriched during surface-associated biofilm development. *lasI* exhibited differential modulation, indicating its regulatory role in coordinating surface behaviour transitions. Importantly, the *lasI* mutant displayed severely impaired swarming and failed to form robust biofilms in the presence of gentamicin, while exhibiting only a marginal reduction in glucose-induced biofilm formation, highlighting the role of quorum sensing in coordinating antibiotic-mediated surface colonization.

**Conclusions:** Our findings show that *P. aeruginosa* adopts a regulated survival strategy by sensing glucose and antibiotic stress to alter surface-associated motility and adhesion. The *lasI*-dependent modulation highlights quorum sensing as a key regulator of adaptive transitions and a potential target to disrupt biofilm resilience.



## P2.14 - BIOGLASS-BASED MEMBRANES INCORPORATING AN IONIC LIQUID FOR CARTILAGE TISSUE REGENERATION

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**Background & Aims:** Articular cartilage has a very limited intrinsic capacity for self-repair due to its avascular and aneural nature, which often leads to progressive degeneration and osteoarthritis. Current clinical approaches mainly focus on symptom management and do not promote the regeneration of functional hyaline cartilage. In this context, biomaterial-based scaffolds have emerged as a promising strategy for cartilage regeneration, providing structural support and promoting cell activity. This work aims to develop biomaterial membranes incorporating 45S5 Bioglass and the ionic liquid 1-Ethyl-3-methylimidazolium hydrogen sulfate [EMIM][HSO<sub>4</sub>]. The goal is to obtain a multifunctional system with improved structural integrity, bioactivity and potential antibacterial performance.

**Methods:** Biomaterial membranes were designed to incorporate 45S5 Bioglass particles and the antibacterial ionic liquid [EMIM][HSO<sub>4</sub>], aiming to obtain a structure suitable for cartilage tissue engineering applications. The membranes were produced using a simple solvent casting method (doctor blade). The material was further evaluated by physico-chemical, morphological, mechanical and biological and microbiological techniques, namely FTIR and X-ray diffraction analyses, SEM observation for morphology and porosity, degradation studies, compressive mechanical testing, cytocompatibility assessment (MTS assay) using chondrocytes, and antimicrobial evaluation through colony-forming units (CFU) assays against *Staphylococcus aureus* and *Escherichia coli*.

**Results:** The membranes were successfully developed and thoroughly characterised in terms of their physicochemical, mechanical, and biological properties, demonstrating their potential as a multifunctional platform for antimicrobial activity and tissue repair capacity. The incorporation of 45S5 Bioglass enhanced bioactivity, while the inclusion of the ionic liquid contributed to effective antibacterial performance.

**Conclusions:** This work proposes a multifunctional biomaterial combining structural support, bioactivity and potential antimicrobial properties for cartilage regeneration, potentially contributing to the development of improved biomaterials for this application.

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### P3.1 - MONITORING INTRACELLULAR ANTIBIOTIC CONCENTRATIONS IN REAL-TIME USING ALLOSTERIC BIOSENSORS

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**Background & Aims:** Antibiotic treatment can fail due to insufficient drug availability at the site of infection or limited accumulation within bacterial pathogens. However, it is poorly understood how antibiotics penetrate infected tissues and complex bacterial aggregates, limiting insights into the mechanisms of treatment failure. Existing methods for antibiotic detection do not allow real-time tracking of unmodified antibiotics directly inside bacterial cells. To address these issues, we developed genetically encoded allosteric biosensors capable of detecting antibiotics inside bacterial cells via microscopy.

**Methods:** To design such biosensors, we applied domain-insertion method developed by Nadler et al. (2016) followed by iterative FACS sorting. Obtained constructs were characterized *in vitro* and applied in several infection models.

**Results:** As a result, we designed genetically-encoded fluorescent allosteric biosensors for two antibiotic classes, trimethoprim and tetracycline, which enable real-time monitoring of antibiotic concentrations inside bacterial cells. The biosensors consist of circularly permuted EGFP linked to the sensory domains DHFR or TetR. To extend this approach to low oxygen environments, we engineered an oxygen-independent trimethoprim biosensor by fusing DHFR to a circularly permuted version of the fluorogenic protein FAST. Using these biosensors, we monitored the antibiotic exposure dynamics of intracellular *Salmonella enterica* during macrophage infection at the single-cell level, and antibiotic penetration into anaerobic regions of *Vibrio cholerae* biofilms, as well as antibiotic availability in microoxic conditions in a human bladder tissue model infected with uropathogenic *Escherichia coli*.

**Conclusions:** These fluorescent biosensors have the potential to be broadly applied for determining antibiotic distributions at infection sites with high spatial and temporal resolution.



### P3.2 - A HIGH-THROUGHPUT, DROPLET-BASED CULTURE METHOD FOR RELIABLE ASSESSMENT OF BIOFILM GROWTH ON VARIOUS SUBSTRATES

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**Background & Aims:** Well plate-based static biofilm culture is widely used for rapid, high-throughput screening of antibiofilm materials. However, conventional methods—such as microtiter plate assays and the Calgary Biofilm Device—are limited by restricted substrate options, incompatibility with many analytical techniques and low reproducibility. Here, we present a droplet-based biofilm culture method in which biofilms develop within a small inoculum deposited on a substrate. The large surface-area-to-volume ratio of the droplet promotes efficient oxygen diffusion, promoting biomass accumulation with high reproducibility. The method is compatible with diverse material surfaces and allows for direct analysis of surface-associated biofilms using various techniques.

**Methods:** Bacterial strains were precultured overnight in LB medium, and cultures were adjusted to an OD<sub>600</sub> of 0.01–0.1. Polydimethylsiloxane (PDMS) and other 6-mm-diameter test material disks were placed in 24-well plates. A 50 µL of inoculum was deposited onto each disk and incubated statically at 37 °C for 6 h. Biofilms were then washed and analyzed using crystal violet (CV) staining, confocal laser scanning microscopy (CLSM), and scanning electron microscopy (SEM).

**Results:** CV staining showed that *P. aeruginosa* biofilm biomass peaked after 6 h of droplet culture on PDMS and declined thereafter. Frequency-distribution analysis indicated substantially lower batch-to-batch variability in biomass than with microtiter plate culture. Direct imaging of the PDMS-associated biofilms by CLSM and SEM confirmed dense bacterial communities embedded within a polymeric matrix. The biofilms demonstrated canonical antimicrobial tolerance, with a 100× MIC concentration of tobramycin failing to eradicate them. Using droplet culture, *P. aeruginosa* rapidly formed mature biofilms on polyurethane, polystyrene, and glass. Additional pathogens—*S. aureus*, *E. coli*, and *K. pneumoniae*—produced robust biofilms with substantially higher biomass than in microtiter plates. Biofilm biomass and distribution in droplet culture were significantly influenced by the substrate properties and dimensions as well as inoculum properties. Furthermore, the droplet culture enabled rapid screening of antibiofilm coatings, where an elastin-like peptide nanocoating and an AgOx-doped TiOx coating reduced *P. aeruginosa* biofilm bio-mass on silicone rubber.

**Conclusions:** The droplet method supports highly reproducible, rapid biofilm formation across multiple pathogens and substrates. It offers a versatile tool for high-throughput biofilm preparation and analysis, enabling efficient evaluation of next-generation antibiofilm materials.



### P3.3 - EVALUATION OF A DEPOLYMERASE-ENCODING BACTERIOPHAGE AGAINST SHIGA TOXIN-PRODUCING ESCHERICHIA COLI BIOFILMS UNDER DYNAMIC FLOW

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**Background & Aims:** Shiga toxin-producing *Escherichia coli* (STEC) is a major foodborne pathogen associated with severe gastrointestinal disease and hemolytic uremic syndrome, particularly in children under 5 years of age. Cattle represent the main reservoir of STEC, and dairy products may become contaminated during milking and processing. In recent years, an increase in STEC infections linked to the consumption of unpasteurized cheeses has been reported. The ability of STEC to form biofilms enhances its persistence in dairy processing environments, representing a challenge for food safety. The extracellular polymeric matrix of STEC biofilms can be rich in colanic acid, which contributes to protection against environmental stress. In this context, strictly lytic bacteriophages encoding polysaccharide depolymerases emerge as a promising biocontrol strategy. This study aimed to evaluate the effect of bacteriophage M1, whose genome encodes a putative colanic acid depolymerase, on mature STEC biofilms.

**Methods:** A dynamic continuous-flow system was assembled to emulate the hydrodynamic conditions of dairy milk pasteurization equipment, operating under continuous flow at room temperature. Four independent chambers were inoculated with  $5 \times 10^9$  CFU/mL of STEC suspension and connected to a peristaltic pump supplying culture medium for 24 h to allow biofilm formation. Subsequently, a bacteriophage suspension ( $1 \times 10^7$  PFU/mL) was added to each chamber and incubated under static conditions for 1 h, after which flow was restored for an additional 16 h. Chambers were then washed and stained with a 1:1 mixture of Acridine Orange and SYPRO Ruby for visualization by confocal laser scanning microscopy.

**Results:** Phage treatment was associated with a visible reduction in biofilm development compared to untreated controls under dynamic flow conditions. In addition, phage exposure limited further biofilm accumulation within the same chamber after 24 h. Confocal microscopy revealed qualitative alterations in biofilm architecture following phage treatment, including changes in bacterial aggregation patterns and biofilm organization, as observed by confocal imaging.

**Conclusions:** Under the experimental conditions evaluated, bacteriophage M1 induced qualitative alterations in STEC biofilm structure under dynamic flow conditions. These observations suggest that bacteriophages encoding polysaccharide depolymerases may interfere with biofilm organization, highlighting their potential as a complementary biocontrol strategy in flow-associated dairy processing environments.



## P3.4 - PSEUDOMONAS AERUGINOSA BIOFILM DYNAMICS UNDER OCULAR OSCILLATING FLOW CONDITIONS

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**Background & Aims:** Bacterial keratitis (BK) remains the fourth leading cause of global blindness, frequently driven by the ESKAPE pathogen *Pseudomonas aeruginosa*. The clinical challenge of BK is exacerbated by the pathogen's propensity to form robust biofilms, which confer significant resistance to conventional antimicrobial therapies. Current models of biofilm development often utilise static or continuous flow conditions; however, these fail to replicate the unique biomechanical environment of the ocular surface. The cornea experiences distinct pulsatile fluid shear stress caused by the tear film being redistributed during the blink cycle. This study aims to characterize how these specific hydrodynamic fluctuations influence *P. aeruginosa* biofilm architecture and development on surfaces mimicking normal, dry eye diseased, and contact lens-wearing corneal environments.

**Methods:** *P. aeruginosa* PAO1 was cultured in M9 minimal medium to a standardized density of 0.8OD<sub>600</sub>. Using the BioFlux 1000Z system, microchannels were inoculated and incubated for one hour to facilitate adhesion. To simulate the ocular environment, pulsatile flow regimes were applied for 24 hours at frequencies corresponding to blink cycles of healthy eyes, dry eye disease, and contact lens wear. Biofilm morphogenesis was captured via time-lapse imaging every five minutes using a Hamamatsu sCMOS camera. Post-experiment, high-resolution z-stacks were acquired. Quantitative analysis of biofilm thickness, surface coverage, and roughness was performed using BioFlux, ImageJ, and Fiji software.

**Results:** Results showed PAO1 biofilm architecture is highly sensitive to pulsatile flow. The high-frequency regime (0.565 Hz), simulating contact lens wear, produced the thinnest biofilms but the highest surface coverage and roughness, indicating aggressive horizontal expansion. In contrast, the low-frequency "staring" regime (0.0089 Hz) resulted in thickest biofilms and reduced surface area, suggesting significant vertical accumulation under suppressed blinking. These findings highlight how mechanical shear dictates structural development.

**Conclusions:** This study demonstrates that traditional flow models may underestimate the complexity of *P. aeruginosa* biofilms in ocular infections. By utilizing microfluidics to replicate the oscillating/pulsating shear of the blink cycle, we provide a more clinically relevant framework for understanding BK. These results highlight the necessity of considering host biomechanics when developing novel anti-biofilm strategies and suggest that ocular surface health (e.g. tear film stability) directly dictates the severity of biofilm-mediated keratitis.



### P3.5 - ELIMINATION OF BACTERIAL BIOFILM MODELS USING BACTERIOPHAGES AND THEIR LYTIC ENZYMES

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**Background & Aims:** Bacterial biofilms are one of the most effective bacterial defence strategies against antibiotic treatment. The cells produce extracellular matrix made of polysaccharides, proteins, extracellular DNA and water. This protective layer inhibits the penetration of antibiotics, host immune cells and supports the development of persistent subpopulations. We aim to propose a treatment solution using natural bacterial killers such as phages and their lytic enzymes.

**Methods:** We picked and tested 9 biofilm-forming strains from clinical isolates of the most common biofilm producers, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. From hospital wastewater samples, new phages were isolated and their genomes were sequenced. Using *in silico* prediction tools we analyzed over 40 genomes to identify potential lytic proteins. In total, 11 endolysins were preselected for cloning, expression and purification.

**Results:** The phages and endolysins were initially tested for strain susceptibility and lytic activity against planktonic cells, followed with *in vitro* testing against bacterial biofilm formations. Assays, such as defining minimal inhibitory concentration (MIC), measurement of optical density decrease, or plate lysis assay were used for this purpose. From the results, we were able to compare their effectiveness and found proofs of synergy, but also antagonism.

**Conclusions:** In our work, we were able to isolate, analyze and produce new phages and lytic enzymes and we proved some of them to be effective against biofilm producing strains in an already created biofilm. In the future, we hope to propose a new way of treatment with a combined therapeutical preparation as a novel way to treat biofilm infections and to push phage therapy further to the clinical application. We kindly acknowledge the financial support of the research project: FW10010112, Technology Agency of the Czech Republic (MB PHARMA)

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### P3.6 - PHAGE-BASED THERAPEUTICS AGAINST BACTERIAL BIOFILMS IN PRECLINICAL STUDIES

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**Background & Aims:** Biofilm formation represents a highly efficient survival mechanism that enables bacteria to withstand antimicrobial therapy. This complex extracellular polymeric matrix acts as a physical and biochemical barrier, limiting antibiotic diffusion, reducing susceptibility to host immune responses and promoting the emergence of persistent bacterial subpopulations associated with difficult-to-treat infections. To address this challenge, we conducted an *in vivo* evaluation of a bacteriophage-based therapeutics as a potential approach for the treatment of biofilm-associated infections.

**Methods:** From wastewater samples, we isolated, analysed, propagated and purified new bacteriophages. From their genomes we identified and produced novel endolysins. After initial susceptibility testing confirming their lytic activity, we moved towards *in vivo* experiment on a biofilm infection murine models, such as biofilm infection of a burn wounds and biofilm associated intestine inflammations. The model infections were treated with phages and endolysins alone and in a combination.

**Results:** In a murine model of TNBS-induced colitis, administration of bacteriophages or high-dose endolysins significantly reduced the expression of pro-inflammatory cytokines and attenuated clinical severity of inflammation. Lower endolysin concentrations had no measurable effect. Neither phages nor endolysins alone substantially reduced intestinal bacterial burden. However, their combination further improved clinical parameters, including reduced weight loss and improved macroscopic inflammation scores. In a murine burn wound infection model, topical administration of phage or endolysin cocktails demonstrated therapeutic efficacy. While phage treatment reduced bacterial load by more than one logarithmic unit, the endolysin cocktail achieved complete elimination of *Klebsiella pneumoniae* from infected burn wounds. In other pathogen-specific infection models we couldn't achieve complete elimination, but we observed reduction in a bacterial burden by three logarithmic units for endolysin cocktail, respectively by two units for phage cocktail.

**Conclusions:** Overall, our findings demonstrate that bacteriophages and their derived endolysins exhibit therapeutic potential against biofilm-associated infections. While their efficacy depended on the infection model, both agents (particularly in combination) could reduce bacterial burden and attenuate inflammatory responses. These results support further development of phage- and endolysin-based strategies as promising biologically driven alternatives for the treatment of difficult-to-treat biofilm infections.

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### P3.7 - DECODING THE EPS MATRIX: A MULTIDISCIPLINARY APPROACH FOR UNDERSTANDING THE RECALCITRANCE OF INDUSTRIAL BIOFILMS

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**Background & Aims:** Biofouling poses significant economic and public health challenges. In cooling systems, biofouling can reduce heat transfer by up to 50% and efficiency by 25%, while simultaneously harboring pathogens that may be disseminated via aerosolization. The recalcitrance of these biofilms is largely driven by the extracellular polymeric substance (EPS) matrix, which acts as a mechanical scaffold and chemical shield. Despite its importance, the matrix remains under-characterized due to its complexity and a lack of repeatable methods that result in quantifiable results. This work aims to develop a multidisciplinary toolkit to characterise the EPS matrix of industrially relevant biofilms and evaluate its response to environmental stressors such as shear flow.

**Methods:** *Klebsiella pneumoniae* ATCC 43816 biofilms were cultivated on stainless steel coupons in microtiter plates at 37°C under 120 rpm agitation for 7 and 14 days. Biofilms were characterised using Optical Coherence Tomography (OCT) for non-invasive structural quantification, rheology to determine mechanical viscoelastic properties, and Confocal Laser Scanning Microscopy (CLSM) utilising specific fluorophores for EPS component targeting.

**Results:** Preliminary OCT analysis revealed a heterogeneous structure with an average biofilm (14-day old) thickness of  $154 \pm 67 \mu\text{m}$  and a porosity of approximately 27%. Rheological characterization demonstrated a dominant elastic modulus ( $G' > G''$ ), confirming the gel-like mechanical behavior of the biofilm matrix. CLSM imaging successfully validated SYPRO Ruby and Calcofluor White as robust stains for visualising the proteinaceous and polysaccharide fractions of the matrix, respectively. Evaluation of specific stains for extracellular DNA (eDNA) is currently ongoing.

**Conclusions:** This study advances the standardisation of EPS matrix analysis by integrating biological, structural, and mechanical datasets. Current work is applying this multidisciplinary framework to correlate the identified matrix architecture with biofilm recalcitrance under varying shear stress conditions. These complementary tools provide a holistic understanding of biofilm architecture and mechanics, establishing a critical foundation for designing more effective, evidence-based anti-biofouling strategies in industrial settings.



### P3.8 - WHY THE BIOFILM FIELD MUST MOVE BEYOND VIABLE PLATE COUNTS: LIMITATIONS AND PATH FORWARD FOR REGULATORY DECISION- MAKING

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**Background & Aims:** Colony-forming unit (CFU) plate counts and viable plate count (VPC) methods have served as the gold standard for microbiological quantification for over a century. Nearly every regulatory guideline—from disinfectant efficacy testing to medical device approval—relies on CFU log-reductions as the primary measure of antimicrobial efficacy. Biofilms represent the dominant microbial lifestyle, and growing evidence suggests that VPC methods fail to capture the structural complexity, physiological diversity, and true microbial burden within these communities. We critically examined the limitations of exclusive reliance on VPC for biofilm assessment and propose a path forward for regulatory decision-making.

**Methods:** a critical analysis of VPC limitations across six key domains was performed: viable but non-culturable (VBNC) cells, incomplete biofilm dispersion, species-specific and polymicrobial cultivation bias, mechanism- of-action blind spots, biomass removal assessment, and antimicrobial resistance detection. Evidence was synthesized from clinical, medical device, water, and food industry contexts.

**Results:** VPC methods miss VBNC cells, which can comprise 10–90% of viable biofilm populations, with molecular methods revealing actual viable counts exceeding VPC by 3–5 log units. Incomplete biofilm dispersion and cultivation bias further compromise accuracy, particularly for polymicrobial and anaerobic communities. Critically, VPC cannot evaluate non-biocidal strategies such as anti-adhesion coatings, dispersal agents, or quorum-sensing inhibitors, as it exclusively measures killing. This creates an uneven regulatory landscape that inadvertently disadvantages innovative approaches to biofilm control—approaches increasingly needed in the era of antimicrobial resistance.

**Conclusions:** VPC data is not incorrect but incomplete for comprehensive biofilm assessment. The path forward requires maintaining VPC as a baseline while incorporating complementary methods—including microscopy, molecular viability assays, and biomass quantification—to enable enhanced, scientifically robust label claims. Regulatory frameworks must evolve to support multi-method assessment that captures the true biofilm state, thereby strengthening public health protection across healthcare, industry, and the environment. In this sense, regulators and decision-makers should consider adopting a tiered guidance model—one that retains VPC as a foundational metric while providing clear, validated pathways for sponsors to supplement or, where scientifically justified, substitute with complementary methods that more accurately reflect biofilm viability and treatment efficacy.



### P3.9 - THE NADH:NAD<sup>+</sup> RATIO INFLUENCES E. COLI K-12 MG1655 SURFACE ATTACHMENT AND BIOFILM GROWTH

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**Background & Aims:** In *E. coli* K-12, curli is the primary adhesin that facilitates surface attachment. The production of curli is regulated by many factors, including low glucose levels and amino acid starvation [1][2]. The global biofilm and curli regulator, *csgD*, is highly transcribed during the stationary phase, which is triggered by nutrient limitation, waste accumulation, and external stresses [3]. This signifies that bacteria can dynamically adapt to metabolic changes by switching to biofilm growth. The NADH:NAD<sup>+</sup> ratio is a parameter that also reflects the bacteria's metabolic and energetic state [4]. In this study, we aim to investigate the link between the NADH:NAD<sup>+</sup> ratio and physiological changes related to biofilm.

**Methods:** The NADH:NAD<sup>+</sup> ratio was altered in *E. coli* K-12 MG1655 using inducible plasmids that overexpressed either the *Candida boidinii* formate dehydrogenase (FDH1; increases NADH) or the *Streptococcus pneumoniae* NADH oxidase (NOX; increases NAD<sup>+</sup>) [5]. We studied the effects of changing the NADH:NAD<sup>+</sup> ratio on: biofilm formation using the crystal violet assay; biofilm morphology using CLSM; swimming motility using soft agar; and curli expression using a *csgD::gfp* fluorescent reporter. We also investigated whether changing NADH:NAD<sup>+</sup> ratio altered membrane potential.

**Results:** An increased NADH:NAD<sup>+</sup> ratio resulted in enhanced biofilm growth, increased *csgD::gfp* activity and reduced flagellar motility. The increased NADH:NAD<sup>+</sup> effect on curli expression was amplified in a mutant strain with a deletion in the PMF-powered AcrB efflux pump, suggesting a link between the NADH:NAD<sup>+</sup> ratio and membrane potential. However, the NADH:NAD<sup>+</sup> alteration showed no significant changes to membrane potential. Imbalanced NADH:NAD<sup>+</sup> could also affect the metabolism through other pathways.

**Conclusions:** This study suggests that the NADH:NAD<sup>+</sup> ratio increased biofilm formation by upregulating curli production, and this potentially occurred via metabolic signalling.

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### P3.10 - MAPPING KEY TRANSCRIPTIONAL SIGNATURES OF *LISTERIA MONOCYTOGENES* PERSISTENCE IN FOOD INDUSTRY BIOFILMS USING A MRNA PNA-FISH STRATEGY

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**Background & Aims:** *Listeria monocytogenes* is a major foodborne pathogen capable of persisting in food-processing environments through biofilm formation. Within these structured communities, the spatial organization of cells generates distinct microenvironments, leading to variability in cellular activity. Consequently, subpopulations emerge with altered gene expression, metabolic profiles, and physiological states, contributing to enhanced tolerance to disinfectants and reduced cleaning efficiency. Therefore, to investigate gene expression within these communities at cellular level, it is essential to maintain biofilm architectural integrity during analysis. This study aims to use a Peptide Nucleic Acid-Fluorescence *in situ* Hybridization (PNA-FISH) approach to localize and analyze key transcriptional signatures related to biofilm development and benzalkonium chloride (BAC) tolerance. PNA-FISH enables *in situ* visualization of gene expression, allowing confocal laser scanning microscopy (CLSM) to reveal spatial heterogeneity within *L. monocytogenes* biofilms.

**Methods:** PNA probes were designed to specifically target the *L. monocytogenes* genes *actA* (biofilm formation), *sigB* (biofilm formation and BAC resistance), and *mdrL* and *sugE2* (BAC tolerance). Fluorochrome selection and hybridization parameters are being optimized to enable multiplex detection and spatial mapping of gene expression within biofilms. Subinhibitory BAC levels were established using MIC assays to simulate disinfectant-induced stress conditions. Gene expression was assessed by RT-qPCR at 24 h and 48 h following BAC exposure.

**Results:** Selected PNA probes showed similar theoretical melting temperatures (65.5-69.6 °C) and favorable Gibbs free energy values (–7.7 to –10.6 kcal/mol), supporting comparable hybridization efficiencies. Fluorophores were pre-selected for future multiplex mRNA PNA-FISH assays (Alexa Fluor 405, Atto 550, Atto 594, and Atto 633), with probe-fluorophore assignment informed by RT-qPCR expression levels. RT-qPCR analysis showed that exposure to 4 µg/mL BAC upregulated target genes: *sigB* increased ~11-fold at 24 h and ~4.5-fold at 48 h, while *actA*, although not directly linked to disinfectant tolerance, was also upregulated (~1.8-fold at 24 h and ~2.3-fold at 48 h), indicating a potential role in BAC tolerance.

**Conclusions:** The upregulation of stress-response genes such as *sigB* emphasizes their importance in bacterial survival under disinfectant exposure in food industry plants. Future studies using spatial transcriptomics will provide detailed insights into biofilm resilience, informing more effective disinfection strategies and enhancing food safety.



### P3.11 - ENZYMATIC BREAKDOWN OF BIOFILM MATRIX TO ALLOW FLOW CYTOMETRY VIABILITY ANALYSIS OF CLOSTRIDIUM BEIJERINCKII CELLS

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1 - IFP Energies nouvelles

**Background & Aims:** Flow cytometry (FC) is a good way to enumerate the number of viable cells in suspension but is not adapted to mature biofilm analysis. The aim of this study is to investigate the effect mechanical treatment coupled with enzymatic hydrolysis of biofilm matrix on FC viability analysis of biofilm cells.

**Methods:** Biofilm was grown for 300 h of continuous fermentation on polyurethane foams. Fermentation was stopped and the biofilm was detached by agitating the foams in PBS buffer with vortex agitation for two minutes. Then, different enzymatic hydrolysis were tested and compared: use of different enzymatic cocktails or pure enzymes. After these enzymatic treatment, biofilm cells detached from polyurethane foams were stained with both propidium iodide (PI) and carboxy fluoresceine diacetate (cFDA) and analyzed by flow cytometry (FC).

**Results:** The best enzymatic hydrolysis consisted of sequential use of DNase I and proteinase K incubated for 1h at 34°C. FC analysis performed after vortex agitation revealed the presence of high non fluorescent events ( $78.9 \pm 3.3$  %). After enzymatic treatment, a cell population was extracted from background noise and could be observed on FSC-SSC profile. The non-fluorescent events of this cell population decreased drastically to  $41.9 \pm 6.6$  % and percentage of viable cells was enhanced from  $2.6 \pm 0.9$  % to  $38.2 \pm 4.0$  % compared to analysis performed after mechanical treatment alone.

**Conclusions:** Consequently, protease and nuclease activity are essential to hydrolyze EPS prior to FC viability analysis in mature biofilm formed by *C. beijerinckii*. This innovative enzymatic treatment allowed the viability analysis of mature biofilm and could help monitor biofilms growth and activity for continuous immobilized cell process.



### P3.12 - EVOLUTION OF BIOFILM CELLS VIABILITY IN FIXED BED BIOFILM REACTOR DURING ISOPROPANOL BUTANOL ETHANOL FERMENTATION WITH CLOSTRIDIUM BEIJERINCKII DSM 6423

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**Background & Aims:** Fermentation using solventogenic *Clostridium* is a promising method for producing butanol and isopropanol from renewable resources. However, its productivity often falls short, making it less competitive than traditional petrochemical processes. To address this, biofilms have been employed to enhance the productivity of various fermentation processes. When applied to isopropanol/butanol fermentation, biofilm technology increased productivity by tenfold compared to batch fermentation. Despite its benefits, biofilm formation is a complex process influenced by numerous parameters, and a high active biomass is crucial for achieving high productivity in immobilized cell systems. While suspended cell viability has been extensively studied using flow cytometry, there is a gap in understanding the viability of *Clostridia* biofilms, which is essential for designing long-term process operations. This study aims to understand how the growth and concentration of both suspended and immobilized cells impact process productivity.

**Methods:** To achieve this, continuous fermentation with cell immobilization was conducted over 528 hours. The dilution rate was gradually increased at a fixed rate of  $2.0 \cdot 10^{-4} \text{ h}^{-2}$  to follow biofilm growth and prevent solvent inhibition. Process performance was evaluated by monitoring glucose, acid, and solvent concentrations over time. Flow cytometry was used to analyze the kinetics of viable immobilized and suspended cells.

**Results:** Throughout the fermentation, productivity steadily increased, reaching a peak of 1.7 g/L/h. Viable cells were present in suspension at dilution rates between  $0.03 \text{ h}^{-1}$  and  $0.07 \text{ h}^{-1}$ , then a washout of viable suspended cells occurred despite high total suspended cells concentration. This viability loss could be partly due to biofilm detachment. The biofilm growth dynamics revealed three distinct developmental stages. Initially, both the percentage of immobilized viable cells and the corresponding concentration were low. During the second phase, the concentration of viable immobilized cells increased sevenfold, and the percentage rose from 0.2% to 41.7%. In the final phase, this percentage dropped sharply from 41.7% to 3.3%, while the concentration of viable immobilized cells remained constant, suggesting that the growth rate of active cells equaled their death rate. This increase in cell death rate might be caused by the high butanol concentration in the culture medium, which reached up to 7.9 g/L.

**Conclusions:** A strong correlation was found between active immobilized cell concentration and process volumetric productivity, indicating that most butanol producers were inside the biofilm than in suspension. Thus, flow cytometry proves to be a valuable method for analyzing *Clostridium* biofilm cell viability, providing insights into the processes occurring during immobilized cell fermentation.



### P3.13 - MULTIPLEXED ANTIMICROBIAL SUSCEPTIBILITY TESTING IN MICROFLUIDIC DROPS

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**Background & Aims:** Antimicrobial resistance (AMR) and biofilm-associated infections represent major and interconnected challenges in clinical microbiology. Bacteria within biofilms exhibit pronounced phenotypic heterogeneity and elevated tolerance to antibiotics, often leading to treatment failure despite susceptibility predicted by conventional antimicrobial susceptibility testing (AST). Current AST methods are slow (18–72 h), population-averaged, and poorly suited to capturing the single-cell variability that underlies both heterogeneous resistance and biofilm persistence. Here, we developed and validated a rapid, high-throughput, single-cell AST platform capable of multiplexed antibiotic testing and quantitative analysis of resistance heterogeneity.

**Methods:** A drop-based microfluidic AST platform was developed using a plate-interfacing, parallel encapsulation (PIPE) chip to generate multiplexed libraries of spectrally barcoded drops. Individual drops encapsulated single *E. coli* cells and defined antibiotic concentrations spanning six antibiotics and up to six dilutions each (36 total conditions). Bacterial growth was quantified using GFP fluorescence and analyzed at kilohertz rates via flow-based laser-induced fluorescence. Gaussian mixture modeling enabled automated discrimination of biotic, non-growing, and growing drops, permitting detection of dormant or weakly growing phenotypes commonly associated with biofilm cells. Population-level drop MICs (dMICs) were extracted from fluorescence dose-response curves and compared with reference broth microdilution MICs (rMICs). Single-cell MIC probability density functions (scMIC PDFs) were derived to quantify resistance heterogeneity and resistant fractions.

**Results:** The platform generated approximately 150,000 single-cell growth measurements per experiment, yielding more than 4,000 single-cell readouts per antibiotic condition within a single multiplexed assay. dMICs showed strong agreement with rMICs for five of six antibiotics, with consistent susceptible/intermediate/resistant classifications. The scMIC PDFs revealed substantial phenotypic heterogeneity within isogenic populations, with resistant fractions ranging from ~21–26% depending on antibiotic. These distributions resemble tolerance patterns reported for biofilm-associated populations. Statistically significant growth differences were detectable within 1 hour of incubation, demonstrating the potential for rapid AST readouts.

**Conclusions:** The multiplexed drop-based AST platform enables rapid, scalable, single-cell profiling of antibiotic susceptibility and phenotypic heterogeneity. By resolving non-growing, tolerant, and resistant subpopulations, the system provides a powerful screening and modeling tool for antibiotic responses. While demonstrated here using planktonic cells, the platform is readily extensible to cells harvested from biofilms, dispersed biofilm fragments, or small multicellular aggregates.



### P3.14 - A BIOLUMINESCENCE QUANTITATIVE ASSAY EVALUATION TO DETERMINE THE BIOMASS OF ATTACHED BACTERIA IN SURFACE BIOFILM FORMATION

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**Background & Aims:** Biofilm-associated infections represent a major clinical challenge due to their increased tolerance to antimicrobial treatments. Consequently, evaluating antibiotic activity against pre-established biofilms is key to optimising therapeutic interventions. Biofilm measurement is challenging due to inherent heterogeneity, complex extracellular matrix structures, and, in clinical settings, a lack of standardised, non-destructive protocols. This study aimed to assess the reproducibility bioluminescence assay and the effect of meropenem on biofilm viability in three clinically relevant bacterial species.

**Methods:** Biofilm formation by *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* was evaluated in a 96-well plate format. For each bacterial species, three independent biological replicates were performed. Within each biological replicate, six technical replicates were analysed: three wells served as untreated controls, while the remaining three wells were treated with meropenem to assess its biofilm-disrupting activity. After treatment, cell viability was quantified using a luminescence-based assay measuring intracellular ATP levels, according to the manufacturer's instructions. Luminescence signals were used as an indicator of metabolically active bacterial cells within the biofilm.

**Results:** Comparable results were obtained across all three bacterial species. Meropenem treatment resulted in a consistent and marked reduction in biofilm-associated bacterial viability, corresponding to a decrease of approximately two orders of magnitude, from an estimated concentration of  $10^6$  to  $10^4$  viable cells. Low variability was observed among technical replicates within each condition, and high reproducibility was confirmed across the three biological replicates for each species. These findings indicate a robust and repeatable response to meropenem treatment in biofilm-grown bacteria.

**Conclusions:** Meropenem demonstrated a similar and significant biofilm-disrupting effect against *E. coli*, *P. aeruginosa*, and *K. pneumoniae* under the tested conditions. The reproducibility observed across both technical and biological replicates supports the reliability of the experimental approach and the suitability of the luminescence-based viability assay for biofilm viability assessment. These results contribute to a better understanding of antibiotic efficacy against biofilm-associated bacterial populations.



### P3.15 - BIOFILM DYNAMICS IN CYSTIC FIBROSIS: APPLICATION OF NAM-FISH IN AIR3GEL, A 3D AIRWAY MUCUS MODEL

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**Background & Aims:** In cystic fibrosis (CF), biofilm growth within airway mucus is a key driver of persistence and treatment resistance in chronic respiratory infections. Advanced *in vitro* models are therefore critical to reproduce CF-relevant conditions and support controlled studies of biofilm behavior and interspecies interactions. This study investigated biofilm formation by three CF-associated pathogens (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*) in a three-dimensional airway model, establishing single-species baselines to enable subsequent comparisons with polymicrobial biofilms and quantify interspecies effects on growth.

**Methods:** Biofilms were grown in Air3Gel, a heterogeneous 3D airway model that mimics key features of the CF lung environment and promotes microcolony aggregates resembling those observed clinically in CF mucus. Bacterial populations were mapped by peptide nucleic acid fluorescence *in situ* hybridization (PNA-FISH) coupled with confocal laser scanning microscopy (CLSM), and complemented with quantitative measurements based on colony-forming unit (CFU) counting and 4',6-diamidino-2-phenylindole (DAPI) staining.

**Results:** In single-species biofilms, CFU-derived viable cell loads decreased from 24 to 72 h across all species, with average reductions of 8.71-fold for *K. pneumoniae*, 2.04-fold for *P. aeruginosa*, and 1.70-fold for *S. aureus* (cells/mL). Concordantly, PNA-FISH quantification together with DAPI staining supported the trends observed in culture-based measurements.

**Conclusions:** Air3Gel combined with PNA-FISH provides a robust framework to quantify and visualize CF-relevant biofilms while capturing their dynamics. By increasing model complexity without compromising experimental control or reproducibility, the platform delivers quantitative outputs that support systematic model evaluation and enable future studies on interspecies interactions and therapy response.



### P3.16 - MRNA PNA-FISH PROFILES SPATIAL GENE EXPRESSION DYNAMICS IN LEGIONELLA PNEUMOPHILA BIOFILMS

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**Background & Aims:** Biofilms are spatially structured microbial communities in which gene expression varies across microenvironments. Peptide nucleic acid fluorescence in situ hybridization targeting mRNA (mRNA PNA-FISH) enables direct visualization and quantification of gene expression at single-cell resolution without disrupting biofilm architecture. This work aimed to establish and quantitatively validate mRNA PNA-FISH as a spatial transcriptomic tool to study the regulatory network involved in the biphasic life cycle of *L. pneumophila*, thereby clarifying the organization and functional development of biofilms.

**Methods:** PNA probes targeting replicative (*secE*), transmissive (*rpoS*, *mip*, *fliA*), and housekeeping (16S rRNA) genes were designed using bioinformatic tools. Probe hybridization conditions were optimized in planktonic *L. pneumophila* serogroup 1 (ATCC™ 33152) grown in buffered yeast extract (BYE) medium. Monospecies biofilms were developed in a continuous-flow cell system. Gene expression levels were quantified by RT- qPCR and correlated with mRNA PNA-FISH fluorescence intensity. Following single-probe validation, multiplex detection was implemented to enable simultaneous visualization of transcripts.

**Results:** Optimal hybridization was achieved at 60°C for all targets. In planktonic cultures, mRNA PNA-FISH fluorescence intensities showed strong linear correlations with RT-qPCR quantification across all genes. Quantitative accuracy was maintained under multiplex conditions despite spectral narrowing ( $R^2 = 0.8891$ ). When applied to monospecies biofilms grown under continuous flow, the method maintained quantitative consistency ( $R^2 = 0.8817$ ). Spatially resolved imaging revealed heterogeneous gene expression patterns, with levels varying across timepoints, reflecting dynamic transcriptional regulation during biofilm development. These results demonstrate that mRNA PNA-FISH enables quantitative, in situ profiling of transcriptional activity within intact biofilm structures.

**Conclusions:** This study demonstrates that mRNA PNA-FISH provides a robust and quantitative approach to investigate spatial gene expression in *L. pneumophila* biofilms. The validated multiplex workflow supports spatiotemporal investigation of transcriptional regulation without disrupting biofilm architecture. Beyond this model system, this approach offers a transferable framework for studying gene expression dynamics in diverse biofilm-based case studies across industrial and clinical settings.



### P3.17 - ANTIBIOTIC-RESISTANT AND TYRAMINE-PRODUCING ENTEROCOCCUS FAECALIS ISOLATED FROM CHEESE FORM ROBUST BIOFILMS ON STAINLESS STEEL

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**Background & Aims:** Biofilms are of particular concern as they may act as reservoirs of antibiotic-resistant bacteria and biogenic amine-producing microorganisms. The accumulation of biogenic amines in cheese, such as tyramine, represents a significant food safety issue due to their potential adverse health effects. Thus, the aims of this study were to characterize the biofilm formation of an *Enterococcus faecalis* strain isolated from a cheese sample with a high tyramine concentration under conditions simulating dairy-processing environments, and to evaluate its resistance to antibiotics and its potential to produce tyramine.

**Methods:** Biofilm formation was evaluated in skimmed milk on 316L stainless steel 24-well plates under static conditions at 37 °C. Sessile cells counts and biomass measurement were quantified over the incubation time (24-96 h) by plate counting and optical density at 595 nm. In order to evaluate its resistance to antibiotics, minimum inhibitory concentrations (MICs) of ampicillin, chloramphenicol, erythromycin, tetracycline and streptomycin were determined for the isolates. The presence of the *tdc* gene was also investigated by PCR to evaluate the strain's potential to produce tyramine. In addition, whole-genome sequencing will be performed de novo to identify target genes.

**Results:** The *E. faecalis* isolate demonstrated a strong ability to form biofilms in skimmed milk on 316L stainless steel plates at the solid-liquid interface. Sessile cell counts exceeded 10<sup>8</sup> cells per well after only 24 h of incubation and remained stable throughout the experimental period. Total biomass increased progressively, reaching a maximum at 72 h. Based on CLSI breakpoints, the strain exhibited resistance to ampicillin and tetracycline, with MIC values eight-fold and two-fold higher than the established thresholds, respectively. Moreover, PCR analysis confirmed the presence of the *tdc* gene, indicating its genetic potential for tyramine production.

**Conclusions:** This study reveals the presence of antibiotic-resistant and tyramine-producing bacteria capable of forming biofilms under dairy-processing conditions, highlighting their persistence and recontamination risks in dairy facilities, compromising food safety, and the potential public health impact associated with antimicrobial resistance and tyramine accumulation.



## P3.18 - FLUORESCENT IMAGING SHEDS LIGHT ON MYCOBACTERIAL BIOFILM ARCHITECTURE IN VITRO AND EX VIVO

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**Background & Aims:** The genus *Mycobacterium* contains various clinically significant, biofilm-forming species, which can cause persistent and often severe infections. *Mycobacterium tuberculosis* alone affects over 10 million people annually. Mycobacterial persistence is likely driven by biofilm formation, since *M. tuberculosis* biofilms have been shown to form *in vivo*. Mycobacterial biofilms have distinctive cording morphology, which has been linked to virulence of *M. tuberculosis*. In this study, we investigated the architecture of mycobacterial biofilms to better understand their role in pathogenicity.

**Methods:** *Mycobacterium marinum* was used as a model organism in this study. It is genetically close to the human pathogen and, as a natural fish pathogen, suitable for *in vivo* experiments. Fluorescent strains were created by introducing plasmids for fluorescent protein expression into selected bacterial strains. Several mutant strains were selected based on observations of altered virulence. Mature *in vitro* biofilms were imaged with confocal microscopy to reveal their 3D architecture. For *ex vivo* imaging, adult zebrafish were infected with fluorescent *M. marinum*. Bacterial granulomas were collected 2–12 weeks after infection. Visual inspection and computational analyses were performed to assess differences in biofilm architectures.

**Results:** The 3D architecture of submerged-type biofilms revealed that *M. marinum* forms cord-like structures *in vitro*. Mutants with lowered virulence exhibited disturbed cording morphology. This suggests a strong link between biofilm architecture and pathogenicity. The *ex vivo* imaging showed maturation of granulomas and presence of known ECM components already at early stages (2 weeks post infection). This indicates that *M. marinum* forms biofilm *in vivo*.

**Conclusions:** Biofilm forming mycobacteria cause persistent, hard-to-treat infections. In this study we showed that the biofilms of *M. marinum* have the distinctive cording morphology that has been previously linked to increased virulence in other mycobacterial species. Using high-resolution imaging combined with computational analysis, we were able to reveal differences in biofilm architectures between various mutant strains with altered virulence. Moreover, we showed formation of *M. marinum* biofilms *in vivo* in an adult zebrafish model. Understanding the structure and organization of mycobacterial biofilm *in vivo* could help the development of more rapid and effective treatment methods against mycobacterial biofilm infections such as tuberculosis.



### P3.19 - MULTISCALE INVESTIGATION OF BIOFILM DYNAMICS AND REMEDIATION IN POROUS MEDIA

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1 - IFPEN

**Background & Aims:** Biofilms play a critical role in subsurface operations, where their presence significantly influences the efficiency and sustainability of geothermal energy extraction, underground gas storage, aquifer storage and recovery and bioremediation. In these environments, microbial communities adhere to mineral surfaces, forming complex structures that can modify permeability and alter fluid chemistry. Understanding their life cycle—from initial attachment to stabilization and eventual removal—is essential for optimizing industrial performance. Through integrated experimental setups, we aim to better understand how porous media characteristics and fluid chemistry impact biofilm dynamics.

**Methods:** By utilizing this complementary experimental framework, we are able to cultivate stable and metabolically active biofilms under controlled flow conditions that mimic subsurface stressors. To capture the complexity of these biological systems, we employ a diverse suite of monitoring techniques. Effluent analysis, including metabolic by product quantification and colony-forming unit (CFU/ml) counts, provides insights into the real-time activity and viability of the consortium in coreflood systems. On the other hand, advanced imaging modalities, such as epifluorescence microscopy and Scanning Electron Microscopy (SEM), allow to characterize the spatial distribution, structural morphology of the biomass and induced precipitations within the pore space.

**Results:** This work presents a comprehensive multiscale framework designed to investigate biofilm behavior across various spatial resolutions, ranging from micrometric microfluidic models (micromodels) to centimetric coreflood systems.

**Conclusions:** The ultimate goal of this research is to refine remediation strategies and enhance the management of microbial consortia, ensuring the long-term viability of geo-engineered reservoirs.



## P3.20 - A TRANSCRIPTOMIC DISPERSAL FINGERPRINT OF PSEUDOMONAS AERUGINOSA

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**Background & Aims:** Biofilm development is a dynamic process comprising surface attachment, biofilm maturation, and dispersal, with each stage characterised by distinct physiological adaptations. While these stages and their molecular drivers have been extensively defined in open-flow systems, they remain comparatively understudied in closed culture platforms widely used for antibiofilm drug screening. Here, we aimed to (1) characterise stage-specific transcriptional profiles of *Pseudomonas aeruginosa* PAO1 across the biofilm life cycle in closed cultures, and (2) identify transcriptional biomarkers of spontaneous biofilm dispersal that can be implemented as robust and scalable dispersal reporter tools.

**Methods:** Biofilm kinetics in closed cultures were quantified by crystal violet biomass staining and microscopy across time-resolved stages, followed by RNA extraction from cells representing attachment, early biofilm maturation, and dispersal. rRNA-depleted libraries were sequenced, mapped to the PAO1 reference genome, and analysed using DESeq2. Candidate dispersal biomarkers were selected by stage-inverse transcriptional signatures, assessed for conservation by BLAST across complete *P. aeruginosa* genomes, validated by RT-qPCR, and implemented as promoter-lacZ transcriptional fusion reporters quantified via  $\beta$ -galactosidase assays.

**Results:** Closed biofilm cultures synchronously recapitulated attachment, maturation, and spontaneous dispersal, hallmarked as a post-peak loss of attached biomass. Attachment was associated with increased transcription of the Pil-Chp surface-sensing machinery, whereas during biofilm maturation matrix-associated pathways were upregulated, including *pel* genes and denitrification. Dispersal was characterised by strong induction of matrix-remodelling and quorum-sensing-linked functions, including rhamnolipid biosynthesis, extracellular nucleases, and *cis*-2-decenoic acid signalling, alongside with marked upregulation of multiple non-canonical phosphodiesterases able to hydrolyse the biofilm secondary messenger cyclic di-GMP. Fourteen robust dispersal-upregulated transcripts that were highly conserved across complete genomes were validated by RT-qPCR. Promoter-lacZ fusions for all biomarkers provided a rapid readout of dispersal-associated transcription, with the majority showing significantly increased  $\beta$ -galactosidase activity during dispersal relative to early biofilm maturation.

**Conclusions:** These data establish closed-culture benchmarks for stage-resolved biofilm transcription in *P. aeruginosa* and identify a conserved panel of dispersal-associated biomarkers. The corresponding reporter plasmids provide a low-cost, scalable platform to detect the onset of dispersal and support screening for dispersal-inducing antibiofilm therapeutics.



### P3.21 - DEVELOPMENT OF A SCREENING STRATEGY FOR BIOFILM-FORMING BACTERIA ISOLATED FROM CHEESE PROCESSING ENVIRONMENTS

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**Background & Aims:** Biofilm formation by dairy-associated bacteria is a concern in cheese production, as it enhances microbial persistence, increases tolerance to cleaning and disinfection procedures, and raises the risk of cross-contamination. The aim of this work was to develop a reproducible methodology for the screening and characterization of biofilm-forming bacteria isolated from cheese samples and evaluated under conditions relevant to the dairy industry.

**Methods:** Cheese samples (n=20) were homogenized, serially diluted, and plated on selective media targeting lactic acid bacteria (LAB), *Enterococcus* spp., and *Staphylococcus* spp. Isolates were screened for biofilm formation using a static incubation assay on 96-well microtiter plates under group-specific growth conditions. To simulate dairy industry conditions, skimmed milk and TSB with 20% sodium chloride (brine-like conditions) were also employed for *Staphylococcus* spp., and skimmed milk for LAB and *Enterococcus* spp. After incubation, biofilms were washed, air-dried, and quantified at 590 nm using a microplate reader in multipoint mode. Biofilm formation was classified according to an optical density cut-off (OD<sub>c</sub>), defined as the mean OD of three negative control plus three standard deviations. Biofilm production was categorized as follows: non-producer ( $\leq$  OD<sub>c</sub>), weak producer ( $>$  OD<sub>c</sub>  $\leq$  2×OD<sub>c</sub>), moderate producer ( $>$  2×OD<sub>c</sub>  $\leq$  4×OD<sub>c</sub>) and strong producer ( $>$  4×OD<sub>c</sub>).

**Results:** 45% of LAB isolates (n=228), 55% of *Staphylococcus* spp. isolates (n=320), and 59% of *Enterococcus* spp. isolates (n=165) were classified as biofilm producers. Among them, 2.94%, 43.75%, and 8.25%, respectively, were categorized as strong biofilm-forming isolates. Thus, although a higher proportion of *Enterococcus* spp. isolates form biofilms, *Staphylococcus* spp. isolates exhibited the highest proportion of strong biofilm producers. When biofilm formation was assessed in milk, a high proportion of LAB and *Enterococcus* spp. isolates were classified as strong biofilm formers compared to their reference growth medium. Otherwise, for *Staphylococcus* spp., milk and salt-supplemented TSB reduced the proportion of isolates classified as strong.

**Conclusions:** This methodology enables systematic screening and characterization of biofilm-forming bacteria from cheese under conditions relevant to the dairy industry. It also allows the identification of robust biofilm producers, which can be employed as standardized models for assessing the effectiveness of cleaning and disinfection strategies against persistent biofilm-forming isolates.



## P4.1 - SYSTEMATIC STUDIES OF BACTERIOPHAGE CONFINEMENT IN GLYCAN-BASED BIOFILMS OF ERWINIA AMYLOVORA

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**Background & Aims:** Biofilms represent a dominant microbial lifestyle in which cells are embedded in a self-produced extracellular polymeric substance (EPS). In many plant-pathogenic bacteria, including *Erwinia* spp., the biofilm matrix is primarily composed of highly viscous, anionic polysaccharides that determine matrix architecture and regulate molecular transport. Despite their ecological and clinical relevance, quantitative insights into how the EPS controls the diffusion of biological nanoparticles, such as bacteriophages, remain limited. This study aims to characterize phage transport in glycan-based biofilm matrices quantitatively and to elucidate how matrix physicochemical properties govern diffusion and infection dynamics.

**Methods:** Polysaccharide matrices were isolated from eight strains (two *Pantoea stewartii*, six *Erwinia amylovora*) and extensively characterized regarding monosaccharide composition, polymer chain length, and production yield. Phage diffusion was investigated using the podovirus  $\Phi$ Ea1h. Fluorescently labeled phages and size-matched microspheres were tracked in purified EPS matrices by single-particle tracking. From particle trajectories, quantitative diffusion parameters and angular distributions were calculated to identify subpopulations with distinct transport behaviors under varying EPS concentrations, pH, and ionic strength.

**Results:** Diffusion dynamics were strongly modulated by EPS concentration and physicochemical conditions. Increased matrix density led to pronounced confinement and hindered diffusion behavior. Comparative analysis of tracer particles and phages revealed size- and surface-dependent transport regimes. Notably, phage-encoded depolymerase activity resulted in rapid stewartan degradation, locally altering matrix structure and significantly enhancing particle mobility. These findings demonstrate that biofilm polysaccharides function as dynamic, size-selective diffusion barriers whose structural and chemical properties critically determine nanoparticle transport.

**Conclusions:** This study provides a quantitative framework for analyzing nanoparticle diffusion in glycan-based biofilm matrices. Our results establish a mechanistic link between EPS physicochemical properties, phage transport dynamics, and infection efficiency. Understanding how biofilm matrix architecture controls molecular mobility advances our ability to predict and manipulate phage-biofilm interactions in environmental and agricultural systems.



## P4.2 - UNDERSTANDING THE IMPACT OF SPATIAL DISTRIBUTION IN BIOFILM COMMUNICATION THROUGH AGENT-BASED MODELLING

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**Background & Aims:** This work presents an 3D agent-based model to observe the impact of the spatial distribution of the cells and cell-cell signalling on the heterogeneity of the biofilm and on the evolution of a polymicrobial community. As a case study, polymicrobial biofilms of *Pseudomonas aeruginosa* and *Candida albicans* were selected, and the impact of the 3D distribution of acyl homoserine lactones (AHLs), a cell-cell signalling molecule produced by *P. aeruginosa*, in the inhibition of *C. albicans* filamentation.

**Methods:** Simulations considered different proportions of species populations, different release rates of AHLs from *P. aeruginosa* and also AHLs release from the boundaries of the environment, to account for the fact that the simulation represented only a small fraction of the entire biofilm. The initial location of the cells in the biofilm was random, and each simulation was repeated three times.

**Results:** As expected, simulation results showed that when more *P. aeruginosa* cells are in the vicinity of a *C. albicans* cell, the likelihood of AHLs reaching *C. albicans* and triggering filamentous inhibition is considerably higher. This difference was more evident when a lower proportion of *P. aeruginosa* cells to *C. albicans* were present. For instance, when *C. albicans* outnumbers *P. aeruginosa* in a ratio of 10 to 1, there can be up to a 5× variation in the amount of time needed for AHLs to reach the individual *C. albicans* cells. On the other hand, when *P. aeruginosa* outnumbers *C. albicans* in a ratio of 10 to 1, the cell-to-cell variation is lower than 2×. Several other aspects of the simulation were also quantitatively assessed, including diffusion coefficients of the individual AHLs molecules or the number of AHLs molecules that collide with *C. albicans* at any time point.

**Conclusions:** Overall, this study demonstrates the importance of spatial location of both cells and molecules in the heterogeneous behaviour of the biofilm. Perhaps more importantly, it provides quantification aspects related to the spatial position that can be useful for better predicting the individual behaviour of molecules and cells in a biofilm.



### P4.3 - A MODULAR COMPUTATIONAL FRAMEWORK FOR MULTIFUNCTIONAL ANTIMICROBIAL PEPTIDE PRIORITIZATION

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**Background & Aims:** The rise of antibiotic resistance worldwide has dictated a swift search for new effective antimicrobial drugs. Antimicrobial peptides (AMP) emerge as promising therapeutic agents since they use multiple non-specific mechanisms of action that are less likely to be circumvented by pathogens. Moreover, therapeutically relevant peptides may display additional key activities such as antibiofilm, immunomodulatory and reduced toxicity to host cells. Unfortunately, AMP discovery is challenged by fragmented experimental data and the need to optimize multiple biological properties simultaneously. This work aimed to establish a computational framework that translates heterogeneous AMP datasets into biologically interpretable modelling axes and supports identification of multifunctional candidates for experimental evaluation.

**Methods:** A unified peptide dataset was constructed by integrating data from multiple public AMP repositories and harmonizing assay metadata. Independent machine learning models were developed for distinct biological dimensions, including antimicrobial potency (covering antibacterial, antifungal, and antiviral activity), safety-related properties, and complementary functional endpoints, such as antibiofilm and antitumor activity. Regression, ordinal, and classification strategies were selected according to data characteristics, with peptide-level grouped validation used to avoid information leakage. Model outputs were organized into multidimensional candidate tables enabling comparison of peptides across individual biological axes.

**Results:** Potency models produced stable peptide rankings and biologically interpretable feature patterns consistent with known AMP physicochemical principles. Comparative analysis of inhibitory and bactericidal models revealed partially distinct feature contributions, supporting separation of biological mechanisms. Integration of safety and functional models enabled multidimensional evaluation of peptides beyond antimicrobial activity alone. The resulting candidate tables provide a structured basis for prioritizing peptides according to different biological objectives, while exploratory biofilm-related modelling demonstrates compatibility of the framework with biofilm-focused applications.

**Conclusions:** This work establishes a modular computational framework that supports multifunctional AMP discovery through machine learning models and multidimensional candidate characterization. The presented baseline framework provides operational outputs for experimental prioritization and serves as a foundation for future integration strategies, including deeper biofilm-focused modelling and broader multifunctional peptide discovery.



## P4.4 - MODELLING THE MECHANICAL RESPONSE OF DRY BIOFILMS UNDER EXTERNAL LOADING: A CONTINUUM APPROACH

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**Background & Aims:** Continuum models have been extensively used to simulate the spatio-temporal dynamics of bacterial biofilms. While these models offer a valuable insight into structural evolution and flow dynamics, they generally rely on simplifying assumptions that may fail to capture biological variability. Biofilm mechanical behavior has also been investigated through viscoelastic or porous models, simulating detachment and deformation in response to physical forces. They are able to provide structural features that are not accounted for in simpler representations. Often, the same mechanical models are studied for both biofilm and tumor microenvironments, neglecting specific biological problems. Nutrient concentrations critically regulate bacterial metabolic activity and govern the transition between growth, dormancy, and stress-induced survival states. The inability to capture these dynamics limits the ability of current modeling frameworks to fully describe biofilm responses. Moreover, dry biofilms have received limited attentions in both experimental and computational investigations. This work proposes a model capable of capturing the coupled biological and mechanical behavior of dry biofilms. The ultimate goal is to investigate, from both modeling and experimental perspectives, their response to externally applied mechanical forces, such as those exerted by a swab during sampling.

**Methods:** This work is framed within continuum mechanics framework rather than an atomistic description. The proposed continuum framework is coupled with a Continuum Bond Method (Sperling et al., 2022) to investigate fracture behavior, enabling the analysis of crack initiation and propagation in dry biofilms. Experimental investigations are ongoing to complement and validate the model.

**Results:** A thermodynamically consistent continuum mechanical framework was developed to describe dry biofilms as a multiphase system. The model relies on mixture theory, in which different species are assigned to two interacting phases. The governing equations were derived from mass and momentum balance principles, with mass balance equations explicitly accounting for the biological behavior of bacterial cells and regulating their dynamics in response to local nutrient concentrations.

**Conclusions:** This work represents a preliminary stage of model development, and numerical simulations are currently ongoing. The proposed framework provides a foundation for investigating the mechanical response and fracture behavior of dry biofilms, with potential implications for improving sampling strategies and understanding biofilm removal mechanisms.



## P5.1 - MODULATING ORAL BIOFILMS THROUGH PREBIOTICS

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**Background & Aims:** Caries and periodontitis are the two most prevalent noncommunicable diseases worldwide, accounting for over 3.5 billion cases (WHO, 2022), driven by microbial dysbiosis. Pathogenic bacteria, such as *Streptococcus mutans*, thrive through increased attachment and biofilm formation, overcoming beneficial commensals like *Streptococcus sanguinis* (Zhu et al., 2018). Interventions that restore a beneficial microbiome and inhibit pathogenic biofilms are key preventative strategies. Prebiotics, defined as substrates selectively utilised by host microorganisms to confer a health benefit, represent a promising therapy. This study aims to investigate the potential of oral prebiotics to modulate the oral microbiome and, consequently, prevent caries and periodontitis. Specifically, we aim to quantify the impact of selected prebiotics on the growth and surface-specific biofilm formation of key oral bacteria, typified by the beneficial *S. sanguinis* and the pathogenic *S. mutans*.

**Methods:** We used *S. sanguinis* and *S. mutans* as model organisms to assess the effect of various prebiotics on microbial growth kinetics and biofilm formation. We quantified biofilm mass and structure established on physiochemically distinct surfaces, mimicking oral tissues, to address the spatial heterogeneity of the oral cavity.

**Results:** Our work to date demonstrates that prebiotics significantly impact the growth and, critically, the surface-dependent biofilm formation of key oral bacteria.

**Conclusions:** Understanding the interplay between prebiotics, bacterial species, and surface characteristics is crucial for developing targeted preventative therapies. This work validates the concept of prebiotic modulation as a viable strategy for restoring a beneficial microbial balance and preventing the world's most common oral diseases. We will present work to date demonstrating the impact of prebiotics on biofilm formation on different surfaces by key oral bacteria.



## P5.2 - EVALUATING LACTAM AS A NOVEL ANTI-BIOFILM STRATEGY FOR ORAL HEALTHCARE.

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1 - Glasgow Caledonian University; 2 - Unilever; 3 - Pro3dure; 4 - Remora Bio

**Background & Aims:** Dental materials including dentures, retainers and mouthguards are prone to colonisation by fungal and bacterial communities which contribute to biofilm formation and microbial dysbiosis. *Candida albicans* interacts with oral bacteria to enhance biofilm resilience, complicating treatment using conventional organism- specific antimicrobials. As such, targeting shared microbial communication pathways represents an attractive therapeutic strategy. This study evaluated the antimicrobial and anti-biofilm activity of a quorum-sensing- derived halogenated furanone (LACTAM-491) against oral-relevant fungal and bacterial species and multispecies biofilm models.

**Methods:** Antimicrobial activity was assessed against a panel of *C. albicans* isolates (n=34) and representative oral bacterial species (n=14), including *Streptococcus mutans* and *Porphyromonas gingivalis*, using planktonic susceptibility testing. Anti-biofilm activity was evaluated using two *in vitro* multispecies oral biofilm models representative of dental caries and periodontitis. Effects on biofilm formation and established biofilms were examined using live/dead qPCR. Ongoing work is using *ex vivo* saliva-derived microbial communities and nanopore-based 16S ribosomal RNA sequencing to assess shifts in microbial community structure following treatment.

**Results:** Planktonic testing demonstrated inhibitory concentrations ranging from 2 to 32 µg/mL across oral-relevant fungal and bacterial species. LACTAM-491 significantly inhibited biofilm formation in both caries- and periodontitis-associated multispecies models. However, treatment did not reduce the bioburden of established biofilms, indicating activity primarily during early stages of biofilm development. Preliminary analyses suggest potential effects on microbial community composition, which are currently under investigation.

**Conclusions:** LACTAM-491 demonstrates antimicrobial activity against oral fungal and bacterial species and inhibits development of multispecies biofilms relevant to caries and periodontitis. These findings support further investigation of quorum-sensing-targeted approaches as strategies to modulate oral biofilm formation.



### P5.3 - MULTISCALE MECHANICAL PROPERTIES OF *S. MUTANS* BIOFILMS

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1 - University of Birmingham

**Background & Aims:** Dental plaque is a structurally and functionally organised stable biofilm, that grows on different substrates and increases the risk of disease due to calcification over time. The knowledge and capability to characterize dental plaque mechanical properties and therefore innovative removal techniques are limited. This work develops a strategy to grow biofilms of *S. mutans* and investigate their physical properties at nano- and micro-length scales.

**Methods:** Ti discs were placed in 6 well plates and a BHI culture with an OD of 0.05 with 1 % sucrose, 0.4 % CaCl<sub>2</sub> was added to the wells. Media was replaced every 24 hours, and 3-day biofilms were generated on Ti discs. For nanoscale mechanical properties, force spectroscopy using an atomic force microscope (AFM) was used; while at the microscale, a custom micromanipulation device was employed in liquid conditions. Young's modulus and adhesion force was extracted from both length scales. A spherical indenter was used at both scales hence a Hertzian model was applied to the raw data. To visualise biofilms grown, confocal laser scanning microscopy (CLSM) was used.

**Results:** AFM allowed for nanoscale mechanical property characterisation by employing a probe with radius of 8 µm. The observed Young's modulus was in the range of 0.64 – 2 kPa for each disc tested. This range was reproducible across 4 biological replicates that were evaluated even with heterogeneity that was observed by CLSM. With micromanipulator, a much larger probe (1 mm diameter) was employed and Young's modulus in the 2 – 10 kPa range was observed across 4 biological replicates. With a larger probe size, the effect of heterogeneity was reduced. Low adhesion forces were observed at both length scales as measurements were conducted in liquid.

**Conclusions:** This work contributes to the multiscale characterisation of dental plaque, examining their physico-chemical properties across length scales and supporting the development of effective plaque removal strategies.



## P5.4 - BIOPHYSICAL CHARACTERISATION OF THE PHYSICAL MODE OF ACTION OF SODIUM BICARBONATE

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1 - Haleon; 2 - SCELSE; 3 - University of New South Wales; 4 - University of Nottingham; 5 - University of Leeds

**Background & Aims:** Dental plaque biofilms are composed of complex microbial communities, which produce a protective matrix that offers mechanical stability and resistance to antimicrobials. These biofilms can lead to oral health conditions such as gingivitis and periodontitis. Clinically, sodium bicarbonate in toothpastes is effective at removing plaque, even in hard-to-reach areas and in-vitro studies have confirmed removal of plaque biofilms, even without brushing. Therefore, the aim of this project was to understand the underpinning action of sodium bicarb treatment on the physical properties of oral biofilms.

**Methods:** Orally relevant biofilm models of differing complexity, including *Streptococcus mutans* monospecies and mixed species communities based on human derived samples, were used across assays. To elucidate the mechanisms of action, we used Confocal Laser Scanning Microscopy (CLSM) with Syto9 staining to assess biofilm biomass and total surface coverage changes, biofluidic microscopy (BFM) to examine adhesion and particle tracking microrheology to evaluate the mechanical properties of oral biofilms. Sodium bicarbonate was used as a raw material at concentrations ranging from 1-67% w/v, or in a toothpaste formulation diluted 1:3 with sterile water.

**Results:** Mixed species biofilms treated with sodium bicarbonate toothpastes showed a marked reduction in total residual biomass and total surface coverage compared to untreated controls (-63.8% and -41.3%, respectively). Particle-tracking microrheology on *S. mutans* biofilms revealed that the elastic modulus increased from ~10 Pa to ~60 Pa after the first treatment, indicating enhanced stiffness due to the removal of the soft upper layer. After 24 hours of regrowth, the elastic modulus decreased to ~20 Pa with a second treatment increasing the modulus to ~50 Pa, 10 Pa lower than the first treatment, indicating a gradual weakening of the biofilm architecture. BFM revealed changes in mechanical properties with a 28% decrease in overall adhesion forces following treatment and single-cell measurements demonstrated decreased adhesion of individual bacteria to treated biofilms ( $5.414^{-9}$  N  $\pm$   $1.190^{-9}$  to  $1.934^{-9}$  N  $\pm$   $5.576^{-10}$ ).

**Conclusions:** Overall, integration of complementary biophysical methodologies provided novel mechanistic understanding for sodium bicarbonate. The results support a physical mode of action, evidenced by biofilm removal, reduced matrix stiffness, and diminished adhesion forces, collectively indicating compromised structural integrity and surface adhesion capacity.



## P5.5 - PARTITIONED LIPID A MODIFICATION PATHWAYS LINK OUTER MEMBRANE VESICLE BIOGENESIS TO BIOFILM DEVELOPMENT AND VIRULENCE IN PORPHYROMONAS GINGIVALIS

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**Background & Aims:** Oral biofilms drive chronic periodontitis, in which *Porphyromonas gingivalis* outer membrane vesicles (OMVs) contribute to matrix architecture, interspecies interactions and host modulation. Lipid A heterogeneity underpins OMV biogenesis and virulence and is generated by multiple modifying enzymes, including the phosphatases LpxE, LpxF1, LpxF2 and the deacylase LpxR. However, whether these enzymes act uniformly or are functionally partitioned between OMV-enriched anionic LPS (A-LPS) and cell-surface O- LPS pools remains unclear. PorR is an essential A-LPS biosynthesis enzyme required for Type IX Secretion System (T9SS) cargo anchoring, including gingipains, and is critical for biofilm maturation. We therefore defined how LpxE, LpxF1, LpxF2 and LpxR differentially regulate lipid A structure, OMV biogenesis, protease export and biofilm development.

**Methods:** Isogenic mutants ( $\Delta$ lpxE,  $\Delta$ lpxF1,  $\Delta$ lpxF2,  $\Delta$ lpxR,  $\Delta$ porR and  $\Delta$ porR $\Delta$ lpxF2) were generated in *P. gingivalis* W50. Lipid A profiles were analysed by MALDI-TOF MS. Membrane stability (SDS sensitivity), gingipain localisation/activity, and biofilm biomass (crystal violet) were measured. OMV and cell ultrastructure were examined by SEM/TEM.

**Results:**  $\Delta$ lpxE lipid A spectra closely resembled  $\Delta$ porR, indicating preferential modification of A-LPS-associated species linked to T9SS cargo export.  $\Delta$ lpxF1 showed similar shifts, supporting a shared role in A-LPS remodeling, whereas  $\Delta$ lpxF2 primarily altered O-LPS-associated lipid A species. Functionally,  $\Delta$ lpxE and  $\Delta$ lpxF1 exhibited markedly reduced OMV-associated gingipain activity and enlarged surface-retained OMVs. In contrast,  $\Delta$ lpxF2 and  $\Delta$ lpxR displayed increased detergent sensitivity with largely preserved vesicle export. Biofilm phenotypes diverged:  $\Delta$ lpxE/ $\Delta$ lpxF1 formed increased biomass, whereas  $\Delta$ lpxF2/ $\Delta$ lpxR showed ~40% reductions.

**Conclusions:** Distinct lipid A modification pathways partition *P. gingivalis* A-LPS and O-LPS functions. A-LPS-linked enzymes (LpxE, LpxF1) are essential for OMV biogenesis and virulence export, whereas O-LPS-linked enzymes (LpxF2, LpxR) maintain membrane integrity. The resemblance of  $\Delta$ lpxE to  $\Delta$ porR lipid A profiles suggests T9SS cargo interactions influence LpxE activity. Dysregulating OMV export alters biofilm architecture and suggests that lipid A plasticity shapes biofilm development by balancing virulence factor deployment and structural cohesion in periodontal biofilms.



## P5.6 - MODELLING MICROBIAL BIOFILMS OF ORAL HEALTH STATES FOR ENHANCED PRE-CLINICAL RESEARCH

Ricarda Hawkins (UK)<sup>1</sup>; Khushy Toor (UK)<sup>1</sup>; **Katie Grey (UK)<sup>1</sup>**; Mark Butcher (UK)<sup>1 2</sup>; Rob Howlin (UK)<sup>1</sup>

1 - Haleon; 2 - Glasgow Caledonian University

**Background & Aims:** Oral bacterial biofilms, like dental plaque, are complex communities that significantly contribute to oral diseases. Due to the high cost of clinical studies and restrictions on using human samples, pre-clinical data is essential for refining experiments and understanding mechanisms of action. This study aimed to establish plaque-like biofilm communities in the CDC Biofilm Reactor model to mimic various oral health states, including health, periodontitis, halitosis, and caries.

**Methods:** Orally-relevant microbial communities were seeded from human plaque, saliva and tongue samples. Specific supplements and environmental conditions were used to promote the growth of organisms relevant to different oral health states, with biofilms grown for up to 10 days in triplicate. The communities were analysed using Confocal Laser Scanning Microscopy (CLSM) with Live/Dead staining, CFU counts, and metagenomics.

**Results:** The results reveal distinct microbial profiles for each simulated oral health condition, with significant variations in microbial diversity and abundance. The biofilm model demonstrates high reproducibility, with consistent CFU counts and microbial compositions across multiple trials. Especially biofilms cultured to represent halitosis and periodontitis revealed high reproducibility ( $R^2$  values 0.82 and 0.95 respectively). Confocal microscopy images showed well-structured biofilms, indicative of successful simulation of the oral environment. Caries-associated biofilms presented with high cell density of coccoid bacteria, while health-associated biofilms presented with a higher abundance of rod-shaped organisms. Metagenomic analysis based on beta diversity with Bray-Curtis distances showed distinct differences in overall species composition between the specific conditions ( $p < 0.01$ , PERMANOVA). Pairwise LEfSe analyses (LDA score  $> 2$ ;  $p < 0.05$ ) identified distinct taxonomic signatures between groups. Various *Streptococcus* spp. and *Veillonella* spp. were collectively enriched in health-associated samples, while caries associated communities were significantly enriched in *Lactocaseibacillus rhamnosus*.

**Conclusions:** This work demonstrated the development of a reproducible and reliable method to successfully mimic oral health states such as health, periodontitis and halitosis in the CDC model with distinct species characteristics and morphologies. This model can support rapid evaluation of new ingredients and formulations, unlocking innovative research in oral health.



## P5.7 - ANTIMICROBIAL AND ANTIBIOFILM EFFICACY OF CHITOSAN-LOADED MONARDA ESSENTIAL OIL AGAINST MONO- AND MIXED-SPECIES ORAL BIOFILMS OF STAPHYLOCOCCUS AUREUS AND CANDIDA ALBICANS

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1 - Aligarh Muslim University

**Background & Aims:** Oral biofilms formed by bacterial-fungal consortia represent a significant clinical challenge due to their enhanced resistance to conventional antimicrobials. We hypothesized that synergistic interaction between Monarda essential oil (MEO) with chitosan nanoparticles (CS-NPs) can be effective against mixed-biofilms. The present study aimed to evaluate the *in vitro* antimicrobial and antibiofilm efficacy of chitosan-loaded Monarda essential oil nanoparticles (CS-MEO-NPs) against mono- and mixed-species oral biofilms of *S. aureus* and *C. albicans*.

**Methods:** Monarda essential oil was encapsulated in chitosan using the ionic gelation method to obtain CS-MEO-NPs. MEO was characterized by GC-MS analysis. CS-NPs and CS-MEO-NPs were characterized for particle size analysis, zeta potential measurement, and FTIR spectroscopy. Antimicrobial activity was assessed by agar well diffusion assay and minimum inhibitory concentration (MIC) was determined according to CLSI guidelines against planktonic cultures of *S. aureus* and *C. albicans*. Biofilm formation in the presence and absence of test agents was quantified by using crystal violet assay in 96-well microtiter plates under mono- and dual-species conditions. Scanning Electron Microscopy (SEM) and Confocal Laser Scanning Microscopy (CLSM) on glass and natural teeth disc surface. Comparative analysis was performed between free essential oil, chitosan nanoparticles (CS-NPs) alone, and the CS-MEO-NPs formulation. Chlorhexidine (CHX) was used as a positive control.

**Results:** CS-NPs exhibited a mean particle size of 353.6 nm with a positive zeta potential of +32.1 mV, indicating good stability. FTIR analysis confirmed successful encapsulation of essential oil within the chitosan matrix. The CS-MEO-NPs formulation demonstrated enhanced antimicrobial activity compared to free essential oil, with lower MIC values against both *S. aureus* and *C. albicans*. Significant inhibition of biofilm was observed in mono-species and mixed biofilms for *S. aureus* and *C. albicans*. SEM and CLSM microscopy observations confirmed structural disruption and reduced biofilm in CS-MEO-NPs treated samples.

**Conclusions:** Chitosan-loaded Monarda essential oil nanoparticles exhibited potent antimicrobial and antibiofilm activity against mono- and mixed-species oral biofilms of *Staphylococcus aureus* and *Candida albicans*. These findings suggest that CS-MEO-NPs could serve as a promising natural therapeutic candidate for the management of oral biofilm-associated infections.



## P5.8 - EX-VIVO ANALYSIS OF SALIVA-DERIVED BIOFILMS REVEALS ANTIFUNGAL INFLUENCE ON BACTERIAL COMMUNITY COMPOSITION IN THE PRESENCE AND ABSENCE OF CANDIDA ALBICANS

**Mark Butcher (UK)<sup>1,2</sup>**; Hafsa Abduljalil (UK)<sup>1,2</sup>; Christopher Delaney (UK)<sup>2</sup>; Jason Brown (UK)<sup>2</sup>; Gordon Ramage (UK)<sup>1</sup>

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**Background & Aims:** *Candida albicans* is well established within the oral microbiome but may function as an opportunistic pathogen in oral dysbiosis. Oral antifungal agents are frequently used to control superficial mycoses and for prophylactic use, yet we have little understanding how these can influence complex microbial communities or potentially drive dysbiosis. This study aimed to better understand how antifungals may influence this complex microbiome.

**Methods:** Saliva from 12 individuals was pooled and used as starter culture for growing *ex-vivo* biofilms  $\pm$  *Candida albicans* (n=3). Biofilms were allowed to mature over 5 days with the addition of clinically relevant concentrations of  $\pm$  fluconazole (FLU) or amphotericin B (AMB), and in the presence of a novel antimicrobial agent (LACTAM). DNA was extracted with propidium monoazide to isolate live cell DNA before full-length 16S rRNA amplification and sequencing via Nanopore® technology and additional bacterial (16S) and fungal (ITS) quantification via qPCR.

**Results:** Data showed that biofilms were altered by the addition of antifungal agents both with and without *C. albicans* presence. Antifungal treatments in complex communities without *C. albicans* revealed significant changes in microbial composition, particularly FLU driving *Staphylococcus aureus* overgrowth and LACTAM driving significant differences in species richness. Additionally, *C. albicans* was seen to contribute to relative stability and tolerance to antifungal treatment.

**Conclusions:** Antifungal agents are often adopted with minimal consideration towards the broader impact on other organisms within the microbiome. We have shown here that antifungals cause alterations to the oral microbiome beyond their target organisms. We also show that when *C. albicans* resides in complex microbiomes, that they are tolerant to antifungal therapy. Together these data have implications for antimicrobial stewardship and collateral damage to off-target populations from antifungal therapy.



## P6.1 - ECOLOGICAL THERAPEUTIC STRATEGY FOR VULVOVAGINAL CANDIDIASIS: SELECTIVE ANTIMICROBIAL EFFICACY OF NATURAL EXTRACT-BASED MUCOADHESIVE GELS IN A SIMULATED VAGINAL MICROBIOTA MODEL

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**Background & Aims:** Vulvovaginal candidiasis (VVC) affects up to 75% of women, with 5-8% experiencing recurrence (RVVC). Rather than systemic immunodeficiency, RVVC is driven by a dysregulated, hyperinflammatory local immune response where mucosal overreaction fails to clear the pathogen, exacerbating tissue damage. While *Candida albicans* dominates via robust biofilm formation, the rise of resistant non-*albicans* species further complicates conventional azole therapy. This study evaluates the efficacy of natural extracts-based mucoadhesive gels against strains of *C. albicans* and *C. glabrata*. Utilizing an in vitro *Lactobacillus* co-culture model, we seek to assess the therapeutic potential of these formulations within a simulated vaginal microbiota environment.

**Methods:** Nine gel formulations, including controls and combinations of pomegranate (R), propolis (P), and sisal (S) extracts, were evaluated. Initially, the Minimum Fungicidal Concentration (MFC) was determined against *C. albicans* SC5314, *C. glabrata* KUE100, and four clinical vaginal isolates. Gels exhibiting MFC were tested against mono-species *Candida* biofilms and co-cultures with *Lactobacillus gasseri* ATCC 33323 in 96-well plates. *Candida* biofilms were grown in RPMI (pH 4.5), while co-cultures were maintained in MRS (pH 6.5). Cell viability was assessed at 24 h, and cultivable cell counts were quantified at 24 h and 48 h.

**Results:** Gels incorporating natural extracts demonstrated formulation-dependent reduction of *Candida* viability, with the nanoemulsified triple formulation (RPS) exhibiting superior antifungal activity. RPS treatment resulted in substantial log<sub>10</sub> CFU reduction, enhanced membrane disruption, and metabolic suppression in both *C. albicans* and *C. glabrata* biofilms. These antifungal effects persisted under co-culture conditions. Conversely, *Lactobacillus gasseri* biofilms maintained elevated CFU counts, minimal membrane damage, and preserved metabolic function following all gel treatments, including RPS, demonstrating selective antimicrobial activity that preserves the simulated vaginal microbiota.

**Conclusions:** The nanoemulsified RPS gel exhibits selective antibiofilm activity against *Candida* spp. while preserving *Lactobacillus gasseri* viability and metabolic function. This microbiome-sparing profile highlights the potential of natural extract-based mucoadhesive gels as an ecological therapeutic strategy for managing biofilm-associated vulvovaginal candidiasis.



## P6.2 - ADVANCED GLYCATION IS A MAJOR PATHWAY FOR EXTRACELLULAR POLYMERIC SUBSTANCES (EPS) PRODUCTION IN ACTIVATED SLUDGE BIOFILMS FROM BIOLOGICAL WASTEWATER TREATMENT

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**Background & Aims:** Activated sludge is arguably the most important industrial biofilm in the world with millions of tons produced globally/yr. Core microbial populations of  $\approx 1,500$  species account for 50% of all bacteria in sludges worldwide. Microbial flocculation in wastewater treatment occurs when bacteria produce extracellular polymeric substances (EPS). Sludge EPS is also additionally increasingly considered as a sustainable recoverable biopolymer resource from wastewater treatment, with several industrial sludge EPS extraction facilities commissioned in Europe. Sludge EPS composition, however, is understood only in vague terms, owing to microbial and molecular complexity. Nonetheless, resolving the identity and production pathway of sludge EPS could further promote sludge EPS as a resource, and improve control of the most fundamental process in biological wastewater treatment (i.e. flocculation).

### Methods:

- Novel EPS extractions
- NMR spectroscopy
- LCMS-MS
- Immuno-based detection and imaging Thermogravimetric
- Model activated sludge biofilm-formers

**Results:** The alkali EPS extraction method used industrially for sludge EPS modified bacterial proteins by Maillard-chemistry. The only protein recoverable from alkali EPS was human keratin, owing to its high cysteine content making it resistant to Maillard chemistry. An alternative EPS extraction method was developed that did not promote the Maillard reaction. Nonetheless, the recovered EPS displayed characteristic 2-D NMR spectrum of an advanced glycation end-product, as produced from the non-enzymatic reaction of a reducing sugar with free amino groups of proteins (i.e. Maillard). Furthermore, the recovered product displayed the same derivative thermogravimetric peaks as the sludge from which it was derived, and the Maillard product standard. Inhibition of advanced glycation in *Zoogloea* spp., a model activated sludge biofilm-former, reduced biofilm formation by 20-100% depending on the advanced glycation reaction targeted. Furthermore, the presence of advanced glycation end-products was confirmed in activated sludge and the model biofilm system, by demonstrating that they inhibited the binding of AGE standards to a human AGE receptor (with high binding specificity for AGEs), and with a positive binding response based on immunomicroscopy using the same receptor.

**Conclusions:** Advanced glycation end-products are abundant in the EPS of activated sludge and other biofilms, and are likely a major contributor to sludge flocculation.



### P6.3 - LURKING IN THE BROILER CHICKENS' ENVIRONMENT: BIOFILM FORMATION BY SALMONELLA INFANTIS AND MULTISPECIES COMMUNITIES

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**Background & Aims:** Salmonella is the cause of the second most reported zoonosis in the European Union. This is mainly caused by consumption of contaminated food, such as broiler meat and eggs. In the EU and Belgium, Salmonella Infantis is frequently found in broiler flocks and known as being persistent in the farm environment and difficult to eradicate. It has been hypothesized that biofilm formation is one of the reasons for this persistence, which was the focus of our research.

**Methods:** S. Infantis isolates (n = 145) originating from the Belgian poultry sector were screened for biofilm formation using the crystal violet staining assay in 96-well plates. After 72 hours at 30°C, isolates were divided into strong, moderate, weak and no biofilm formers. Similarly, multispecies biofilms combining S. Infantis with dominant flora from broiler houses (Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Enterococcus faecalis and Staphylococcus arlettae) were assessed. Dual, triple and quattro species combinations were made for both a weak and strong S. Infantis biofilm former.

**Results:** Most S. Infantis isolates, 69.7%, produced weak biofilms, only one isolate formed a strong biofilm. One S. Infantis weak (OD<sub>595nm</sub> = 0.12 ± 0.04) and the strong biofilm former (OD<sub>595nm</sub> = 0.52 ± 0.11) were tested in multispecies biofilms. In dual species combinations, synergy was observed between the weak S. Infantis biofilm former and Staphylococcus (OD<sub>595nm</sub> = 0.84 ± 0.12) and Enterococcus (OD<sub>595nm</sub> = 0.64 ± 0.09). Combined with Pseudomonas or Stenotrophomonas an antagonistic relationship was found. For the strong S. Infantis biofilm former, all dual species combinations resulted in a decreased biofilm formation. In triple species biofilms, the combination of S. Infantis with Staphylococcus and Enterococcus did not result in synergy, contrary to what was expected from the dual species. All other combinations, triple as well as quattro species combinations, showed no synergy in biofilm formation.

**Conclusions:** Mono species biofilm formation of S. Infantis does not explain its persistent nature. In combination with dominant flora of the broiler houses, stronger biofilms were formed, only in limited cases synergy was found. More research concerning the behavior of S. Infantis combined with dominant flora in broiler houses could elucidate its persistent nature.



## P6.4 - KINETIC CHARACTERIZATION OF MONO AND POLYMICROBIAL BIOFILM FORMATION BY CANDIDOZYMA AURIS AND ASSOCIATED BACTERIA

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**Background & Aims:** This study aimed to develop and characterize a monospecific *Candidozyma auris* biofilm in association with commensal (*Staphylococcus epidermidis*) and pathogenic (*Pseudomonas aeruginosa*) bacteria, and to evaluate biomass production, metabolic activity, and structure using scanning electron microscopy.

**Methods:** Biofilm formation was assessed using a clinical isolate of *C. auris*, a clinical isolate of *P. aeruginosa*, and the *S. epidermidis* ATCC 14990 strain. The fungus was cultured overnight on Sabouraud-Dextrose agar to obtain blastoconidia, and a suspension was adjusted to  $1 \times 10^6$  spores/mL. The biofilm was formed in RPMI-1640 medium and incubated at 37 °C. The bacterial species were cultured overnight on tryptone soy agar and a suspension was prepared at  $1 \times 10^8$  cells/mL in RPMI-1640 medium. Characterization was performed every 24 hours to evaluate metabolic activity and biomass formation using the tetrazolium salt (XTT) and crystal violet 0.5%. For scanning electron microscopy, fixation was performed using 2.5% glutaraldehyde and 4% paraformaldehyde, followed by incubation at 4 °C for 1 hour.

**Results:** Regarding single-species biofilms, *C. auris* was able to form a robust and mature biofilm in 96 hours of incubation, while *P. aeruginosa* and *S. epidermidis* formed mature biofilms within 48 h and 24 h, respectively. Concerning polymicrobial biofilms of bacteria and *C. auris*, synergistic activity was observed in the presence of *S. epidermidis*, evidenced by greater biomass production and metabolic activity. The dual-species biofilm between *C. auris* and *P. aeruginosa*, in turn, exhibited antagonistic activity, as no significant increases were observed in the evaluated parameters relative to the single-species biofilms of each microorganism.

**Conclusions:** Therefore, based on the results, *C. auris* forms a slower and more robust biofilm when compared to other species of the genus *Candida* after 96 hours. In contrast, biofilms of *S. epidermidis* and *P. aeruginosa* form more rapidly. Through different combinations of bacterial species, it was possible to observe changes in the metabolic behavior and biofilm formation of *C. auris*. Moreover, scanning electron microscopy images showed a considerable volume of matrix in monospecies *C. auris* biofilms and in association with bacterial species. Further studies on the mechanisms of biofilm regulation are necessary.



## P6.5 - EXPLORING THE MYCOBIOME OF DUTCH DENTAL UNIT WATERLINES

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**Background & Aims:** Dental unit waterlines (DUWS) are highly susceptible to biofilm formation, and biofilm fragments can become aerosolized during use, posing infection risks to patients and dental staff. Recent studies show that each DUWS hosts a distinct bacteriome, often containing *Legionella* spp. and their amoebal hosts. However, far less is known about the fungal component of these biofilms. Our aim was to explore the fungal community of dental unit waterlines.

**Methods:** DUWS effluent samples (n=213) and background info on the cleaning regimens were collected during a fieldstudy. Samples were processed for DNA analysis. The fungal 18S rDNA load was estimated using FungiQuant PCR. ITS2 amplicons were generated purified, indexed, and pooled prior to quality assessment. Paired end sequencing was performed, sequencing reads merged and quality filtered in USEARCH, clustered into 97% OTUs, and mapped to their OTU centroids. Taxonomy was assigned to the most abundant sequence per OTU using the RDP classifier with the UNITE v9.0 database, and samples with <1,000 total reads were removed.

**Results:** Fungal rDNA concentrations were highest in DUWS that received periodic shock dosing (13.7 pg/μl) and lowest in units treated with both a daily low dose and periodic shock dosing (2.23 pg/μl). Untreated units contained 5.39 pg/μl fungal rDNA. After quality filtering of the mycobiome data, 90 samples remained, yielding a total of 1,018,327 reads. The dataset showed high sparsity, with 464 OTUs (>100 reads per OTU) detected; 56% of these could only be taxonomically assigned at the kingdom level. Each DUWS contained on average 10 OTUs (range 1–31; median 8). Heatmap analysis revealed that every dental unit harboured a unique mycobiome composed of plant-associated and environmental fungi as well as opportunistic pathogens. The most prevalent and abundant fungal genera (>50,000 reads) included *Malassezia* spp. (64/90 units), *Cladosporium* spp. (21/90), *Graphium* spp. (19/90), *Neocosmospora* spp. (11/90) and *Exophiala* spp. (10/90), an opportunistic black fungal pathogen.

**Conclusions:** Dental units harbour a unit specific mycobiome in which fungi form an integral component, comprising both environmental species and opportunistic pathogens. This fungal load can be effectively managed by applying a continuous daily treatment in combination with a weekly high dose antimicrobial regimen.



## P6.6 - TARGETING BACTERIAL BIOFILMS USING CORDYCEPS MILITARIS- MEDIATED GOLD NANOPARTICLES: EPS DISRUPTION AND ROS- DRIVEN MECHANISMS

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**Background & Aims:** Biofilm-associated infections significantly contribute to antimicrobial resistance (AMR) by protecting bacterial communities within extracellular polymeric substance (EPS) matrices, leading to persistent and treatment-resistant infections. *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* are major biofilm-forming pathogens implicated in chronic and device-associated infections. The present study aimed to synthesize gold nanoparticles (AuNPs) using *Cordyceps militaris* through a green approach and evaluate their antibiofilm efficacy, supported by mechanistic and in silico molecular docking analyses.

**Methods:** Gold nanoparticles were biosynthesized using aqueous extract of *Cordyceps militaris* as reducing and stabilizing agent. Characterization was performed using UV-Visible spectroscopy, TEM, XRD, and FTIR analyses. Antibacterial and antibiofilm activities were assessed through agar well diffusion, MIC, MBC, crystal violet biofilm assay, Congo red agar assay, motility and swarming assays, ROS detection (DCFH-DA), and EPS quantification (carbohydrate and protein estimation). Additionally, in silico molecular docking was conducted to evaluate interactions between bioactive constituents and bacterial target proteins.

**Results:** UV-Vis spectroscopy confirmed nanoparticle synthesis with a surface plasmon resonance peak at 555 nm, while TEM revealed predominantly spherical nanoparticles (~25 nm). CM-AuNPs demonstrated potent antibacterial activity (MIC: 50 µg/mL). Importantly, biofilm inhibition was pronounced, with >80% reduction in biofilm biomass at higher concentrations. Crystal violet and Congo red assays revealed significant disruption of biofilm architecture and reduced EPS production. Quantitative analysis showed marked decreases in both carbohydrate and protein components of EPS, confirming matrix destabilization. CM-AuNP treatment significantly suppressed *P. aeruginosa* motility and swarming, indicating inhibition of virulence traits essential for biofilm establishment. ROS assays revealed a 15–22-fold increase in intracellular oxidative stress, suggesting ROS-mediated biofilm disruption. Molecular docking demonstrated favorable binding affinities with key bacterial proteins, supporting the mechanistic basis of biofilm inhibition.

**Conclusions:** The study demonstrates that *Cordyceps militaris*-mediated gold nanoparticles effectively disrupt biofilm formation through EPS degradation, ROS induction, and virulence suppression. Integration of green nanotechnology with computational validation highlights CM-AuNPs as a promising strategy for targeting biofilm-associated multidrug-resistant infections.



## P6.7 - ACTIVE PLA-CHITOSAN COATINGS TO PREVENT FOOD-RELATED MULTISPECIES BIOFILMS

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**Background & Aims:** Multispecies biofilms formed by foodborne and opportunistic bacteria persist on food-contact surfaces and exhibit increased tolerance to sanitation procedures, posing a public health concern. *Escherichia coli* and *Pseudomonas putida* can survive under refrigeration, facilitating cross-contamination and foodborne outbreaks. Antimicrobial surfaces capable of preventing biofilm development are urgently needed. This study evaluated the antibiofilm performance of non-chemically modified chitosan extracted from *Loligo opalescens* pens, including depolymerized derivatives with different molecular weights, applied as coatings on Poly(Lactic Acid) (PLA) films.

**Methods:** Chitosan was extracted from *L. opalescens* pens and depolymerized to obtain derivatives with distinct molecular weights, characterized by <sup>1</sup>H Nuclear Magnetic Resonance spectroscopy and Gel Permeation Chromatography. PLA films were plasma-treated and coated with 1% (w/v) chitosan by dip coating. Dual-species biofilms of *E. coli* and *P. putida* were formed on coated and uncoated surfaces at 5 °C for 24 h. Biofilm culturable cells were quantified by plate counting (Colony-Forming Units), while fluorescence staining assessed total and viable cells. Biofilm biovolume and thickness were analyzed by Confocal Laser Scanning Microscopy (CLSM), and morphology by Scanning Electron Microscopy (SEM).

**Results:** Chitosan immobilization reduced the water contact angle of PLA from ~70° to 36–40° without affecting surface roughness. All coated surfaces decreased biofilm total cells by 56% compared to PLA, indicating antibiofilm activity. Depolymerized chitosans reduced viable cells by 67–74% and culturable cells by 70–76%, whereas native chitosan reduced culturability by 66%. On PLA, *E. coli* represented 67% of culturable cells, while *P. putida* decreased to 5–17% on coated surfaces, demonstrating strain-dependent susceptibility. CLSM revealed a 33% reduction in total biovolume and a 43% decrease in biofilm thickness. *P. putida* biovolume decreased by up to 87%, whereas *E. coli* remained constant. Approximately 64% of cells on depolymerized chitosan surfaces were non-viable. SEM showed reduced colonization and membrane damage, supporting electrostatic interactions between positively charged chitosan and negatively charged bacterial envelopes.

**Conclusions:** By limiting multispecies biofilm establishment, this approach mitigates contamination risks while supporting marine waste valorization within a circular economy framework. The demonstrated antibiofilm and bactericidal efficacy of chitosan coatings suggests potential application in other high-risk settings, including medical surfaces.



## P6.8 - DEVELOPMENT OF A PSEUDOMONAS AERUGINOSA AND STAPHYLOCOCCUS AUREUS DUAL-SPECIES BIOFILM MODEL AS A SCREENING PLATFORM FOR BIOFILM DISPERSION ACTIVITY

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**Background & Aims:** *Pseudomonas aeruginosa* (*Pa*) and *Staphylococcus aureus* (*Sa*) are opportunistic pathogens frequently co- isolated from chronic biofilm-related infections. Their coexistence within polymicrobial biofilms enhances increased tolerance to antimicrobial treatments and host immune defenses leading to poor clinical outcomes. The use of exogenously added cues to promote biofilm dispersion has emerged as a promising strategy, as dispersed bacteria revert to the planktonic state and lose the biofilm-associated tolerance to antimicrobials. However, establishing an *in vitro* biofilm model of these two pathogens remain technically challenging, limiting mechanistic and therapeutic investigations. This work aims to establish and optimize a *Pa-Sa* dual- species biofilm model to provide a screening platform for biofilm-dispersing agents.

**Methods:** In this work we developed and optimized an *in vitro* dual-species *Pa-Sa* biofilm model designed for its use as a screening platform for biofilm dispersing agents. Strain pairs displaying a coexistence phenotype were selected from a panel of reference and clinical isolates using an agar-based interaction assay. Biofilm formation was evaluated under different growth conditions and supports. *Pa* and *Sa* growth and distribution within dual-species biofilms were quantified by colony-forming unit (CFU) enumeration on selective media, while total biomass was assessed using crystal violet staining.

**Results:** An *in vitro* *Pa-Sa* dual-species biofilm model was developed on two different supports ; a classic flat-bottom microtiter plate or a device composed of a lid with pegs. Counting on selective medium revealed the presence of the two pathogens at equivalent proportions when grown for 24h in TSB medium. This dual- species biofilm model is currently used as a screening platform to assess a chemical library of *N*-arylimino-1,2,3-dithiazole derivatives compounds for their potential dispersal activity. In a recent study, we showed that these compounds induced significant dispersal activity on *Pa* (PA14 strain) mono-species biofilms. These compounds of interest demonstrated no cytotoxicity on relevant eukaryotic cell models. Moreover, the compounds did not exacerbate *Pa* virulence through pyocyanin production.

**Conclusions:** We established a stable *in vitro* *Pa-Sa* dual-species biofilm model to use as a high-throughput screening platform to evaluate potential antibiofilm agents. Further efforts are needed to develop a more clinically relevant *Pa-Sa* dual-species biofilm model including its interaction with the host.



## P6.9 - THE AGGREGATE ADVANTAGE: UNRAVELLING COMPETITIVE DYNAMICS IN MIXED SPECIES BIOFILMS

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**Background & Aims:** Bacterial aggregates are increasingly recognised as a third lifestyle in the biofilm developmental cycle, distinct from planktonic single cells and surface attached biofilms. While aggregates exhibit unique biofilm forming capacities, few studies have investigated how an aggregated state might dictate competitive success and spatial positioning in complex communities. This study characterised dual species biofilm development and how the introduction of aggregates might affect competition dynamics for *Pseudomonas fluorescens* and *Pseudomonas putida*.

**Methods:** Using microfluidic flow cells and confocal imaging, we examined the growth kinetics and development of single and dual species biofilms. We further introduced size filtered aggregates during flow cell inoculation to examine their altered competitive dynamics. Deep learning based single cell segmentation, combined with particle tracking microrheology was employed to map spatial interaction networks, allowing us to correlate localized competitive outcomes with biophysical phenomena, including single-cell nematic alignment, localised creep compliance and matrix component profiles. Global cellular and matrix architectural characteristics were quantified via 3D biovolume and morphological metrics. We further investigated altered resilience to mechanical stress by inoculating aggregates and planktonic cells in the presence of increased shear.

**Results:** Aggregate inoculation altered biofilm architectures compared to standard planktonic inoculation, with significant modifications to global biomass and thickness. At the single cell scale, deep learning segmentation and microrheology revealed unique profiles in nematic order, cell spacing, and matrix mechanics within aggregate derived communities. Crucially, when competing in a dual species context, aggregates displayed distinct competitive capabilities compared to their planktonic competitors and an altered capacity to persist on the surface despite shear challenge.

**Conclusions:** Understanding bacterial aggregation as an important facet of the biofilm developmental cycle is crucial across environmentally relevant dual species biofilm models. These findings demonstrate that the aggregate lifestyle significantly alters competitive fitness, structural resilience, and spatial dominance during surface colonisation. Such insights facilitate a deeper understanding of biofilm dynamics in environmental and healthcare scenarios.



## P6.10 - BRIDGING THE TRANSLATIONAL GAP: EVALUATION OF A NOVEL DUAL-DRUG ANTIMICROBIAL COATING FOR ENDOTRACHEAL TUBES IN A PHYSIOLOGICALLY RELEVANT QUASI-IN VIVO AIRWAY MODEL

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**Background & Aims:** Endotracheal tubes (ETT) are the primary risk factor for ventilator-associated pneumonia (VAP) due to rapid biofilm formation. While antimicrobial coatings often perform well in vitro, conventional assays poorly replicate biological complexity of the intubated airway, limiting clinical translation. This study aimed to validate the efficacy of our previously developed dual-drug ciprofloxacin/chlorhexidine (CIP/CHX) ETT coating using a physiologically relevant quasi-in vivo airway model designed to closely approximate clinical conditions.

**Methods:** Coating efficacy was strengthened in an adapted quasi-in vivo airway model reproducing key features of the intubated trachea, including anatomical orientation, nutrient limitation, polymicrobial colonization, and prolonged exposure. ETT tubular segments were inserted into a semi-solid, nutrient-restricted matrix mimicking airway mucus and incubated for 5 days (37 °C). Prior to tube insertion, the matrix surface was inoculated with single- or dual-species suspensions pairing *Pseudomonas aeruginosa* with *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, or *Candida albicans*. Biofilm burden was quantified within the ETT lumen and across the total ETT surface.

**Results:** The CIP/CHX coating significantly inhibited biofilm formation under mono- and polymicrobial conditions. This effect was more pronounced on the ETT intraluminal surface, where all single-species biofilms were fully prevented. Likewise, complete inhibition was achieved for the *P. aeruginosa*/*K. pneumoniae*, *P. aeruginosa*/*S. epidermidis*, and *P. aeruginosa*/*C. albicans* dual-species consortia within the lumen. In combinations with *A. baumannii* or *S. aureus*, partner species were fully suppressed, whereas *P. aeruginosa* remained the most resilient organism, although still significantly reduced (3.34-7.38 log<sub>10</sub> CFU/mL). Across the entire ETT surface, polymicrobial populations of *K. pneumoniae*, *S. aureus*, and *S. epidermidis* were significantly reduced, with *P. aeruginosa* overall reductions ranging from 0.75 to 2.27 log<sub>10</sub> CFU/mL. No clinically significant CIP tolerance emerged after 5-day exposure.

**Conclusions:** These findings demonstrate the strong translational potential and sustained antimicrobial performance of our ETT coating under clinically relevant conditions. The implemented quasi-in vivo airway model establishes a robust, physiologically informed and predictive preclinical benchmark that may accelerate development, improve translational confidence, and de-risk next-generation ETT anti-biofilm technologies aimed at preventing VAP.



## P6.11 - NUCLEIC ACID MIMICS - FLUORESCENCE IN SITU HYBRIDIZATION (NAMS-FISH) COMBINED WITH SPECTRAL IMAGING (SI) FOR DETECTION AND CHARACTERIZATION OF GASTRIC MICROBIOTA

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**Background & Aims:** The three-dimensional organization of the gastric microbiota, which may significantly influence physiology and overall health status, remains insufficiently characterized. While previous studies have focused mainly on identifying the taxonomic composition of gastric microbial communities, little is known about how these microorganisms are spatially arranged within the gastric mucosa and how this organization may affect microbial interactions and disease development. In this study, Spectral Imaging (SI), combined with Nucleic Acid Mimics Fluorescence in situ Hybridization (NAMS-FISH), was developed to investigate the spatial distribution of clinically relevant gastric pathogens in complex microbial communities.

**Methods:** Seven genus-/species-specific LNA/2'-OMe probes were designed to target *Campylobacter* spp., *Clostridium* spp., *Citrobacter* spp., *Fusobacterium* spp., *Lactobacillus* spp., *Streptococcus* spp., and *Helicobacter pylori*. Hybridization conditions for each probe—including composition of the hybridization solution, temperature, and incubation time—were individually optimized. The specificity and sensitivity of each probe were subsequently assessed. After individual optimization, a single set of hybridization conditions compatible with all probes was established. The method was then validated through the analysis of defined microbial mixtures containing known and varying concentrations of each genus/species. Spectral imaging was performed using confocal laser scanning microscopy to allow precise fluorophore discrimination and analysis.

**Results:** The optimized hybridization condition selected for the probe mixture consisted of an hybridization solution containing 0.5 M urea, 900 mM NaCl, 50 mM Tris-HCl, 5 mM disodium EDTA, 10% (w/v) dextran sulfate, 0.1% (w/v) sodium pyrophosphate, 0.2% (w/v) polyvinylpyrrolidone, 0.2% (w/v) Ficoll, 0.1% (v/v) Triton X-100, pH – 7.5, a hybridization period of 60 minutes and a temperature of 64 °C. Subsequently, we successfully validated the method, demonstrating a good correlation between the known bacterial input in the mixtures and the quantitative output obtained by FISH analysis.

**Conclusions:** This work establishes a robust methodological framework for studying the three-dimensional spatial organization of gastric microbiota. It provides a valuable tool for characterizing microbial profiles associated with health and disease in adult patients.



## P6.12 - TOWARDS AN UNDERSTANDING OF THE FORMATION OF MIXED- SPECIES BIOFILMS IN THE FOOD PROCESSING INDUSTRY: TEMPERATURE EFFECTS ON BIOFILM DEVELOPMENT IN *LISTERIA MONOCYTOGENES*, *PSEUDOMONAS FLUORESCENS*, *ESCHERICHIA COLI*, AND *SALMONELLA TYPHIMURIUM*

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1 - University of Lincoln

**Background & Aims:** Microbial biofilms pose a significant challenge in food processing environments due to their persistence on surfaces, resistance to traditional disinfection methods and substantial contribution to food contamination and spoilage, leading to increased risks to food safety and public health. Biofilm formation can be influenced by environmental conditions commonly associated with food processing environments, including temperature. We have investigated how biofilms form using different single species and combinations of *Listeria monocytogenes*, *Pseudomonas fluorescens*, *Escherichia coli*, and *Salmonella typhimurium* and to what extent mixed species biofilms are influenced by varying ambient temperatures.

**Methods:** *Listeria monocytogenes*, *Pseudomonas fluorescens*, *Escherichia coli*, and *Salmonella typhimurium*, reference strains alone and in combination, were selected for their relevance to food safety and spoilage. Biofilm formation was assessed using microtiter plate assays. Temperature-dependent biofilm formation was evaluated at 25°C and 30°C, and incubated for 72 hours to reflect temperatures relevant to food processing, storage and optimal bacterial growth. Biofilm biomass was quantified using the crystal violet staining technique.

**Results:** Results indicate that biofilm formation is strongly influenced by temperature, with variations in biomass among the four bacterial species. *Salmonella* showed greater biofilm biomass at 25°C (OD=0.431) compared to 30°C (OD=0.362). In contrast, *E. coli* and *Listeria* showed reduced biomass at 25°C (OD=0.225) to 30°C (OD=0.277) and (OD=0.172) at 25°C to (OD=0.197) at 30°C, respectively. *Pseudomonas* maintained strong biomass (OD=0.421) at 30°C and (0.397) at 25°C. In dual-species biofilms, overall biomass was lower compared to mono-species biofilms. The *Pseudomonas-Salmonella* combination produced strong biofilm biomass at 30°C (OD=0.183) and moderate at 25°C (OD=0.156). The *Salmonella-E. coli* dual-species biofilm at 30°C had (OD=0.187). These results highlight the influence of temperature on bacterial surface attachment and growth.

**Conclusions:** These findings establish a strong methodology for understanding how temperature fluctuations in food processing environments may affect biofilm persistence and contamination risk. Understanding the effect of temperature on biofilm formation is essential in creating effective sanitation strategies and environmental controls within food production industries.



## P6.13 - COMPARATIVE CROSS-SCALE IMAGING OF MONO- AND DUAL- SPECIES BIOFILMS REVEALS SPECIES-DEPENDENT INTERNAL ORGANISATION

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**Background & Aims:** Biofilm growth is an emergent property and frequently unpredictable, particularly in multi-species communities. *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Escherichia coli* are well studied in monoculture, yet exist as polymicrobial biofilms in clinical, environmental and industrial settings, including catheters, the airways of cystic-fibrosis patients, and wastewater systems. Studying these complex bacterial communities requires imaging approaches capable of resolving both cellular detail and large-scale organisation. In this work, we employed cross-scale and multi-modal imaging strategies to examine the internal architecture of bacterial biofilms.

**Methods:** Intra-colony channels, first described in *E. coli* macrocolony biofilms, can transport exogenous materials and distribute nutrients throughout the biomass. However, it is unknown whether these channels are conserved across diverse bacteria. By combining laser-scanning and spinning-disk confocal microscopy, and mesoscopy with the Mesolens, we have expanded on previous studies to visualise biofilm structures across a range of spatial scales. These cross-scale and multi-modal imaging techniques enabled enhanced depth, speed and more sensitive imaging than conventional approaches, improving our ability to resolve intricate biofilm structures and cell-cell interactions in phylogenetically distinct bacteria.

**Results:** Using three converging cross-scale imaging techniques, we visualised transport channels embedded within *P. aeruginosa* biofilms and spatial variation in mixed biofilms that could not be identified by one imaging technique alone. Additionally, functional assays revealed that these structures act to uptake physical nanoparticles from the environment, which provides evidence for a possible nutrient pathway system. Comparative imaging of mono- and dual-species biofilms showed that *P. fluorescens* occupies the transport channel regions of the *E. coli* biomass, while *P. aeruginosa* and *E. coli* remained spatially separate with well-defined borders.

**Conclusions:** Current work focuses on understanding the effect of nutrient, chemical and environmental stressors within these structures and developing quantitative approaches to compare biofilm architecture in single species and polymicrobial biofilms. Additionally, investigating clinically relevant isolates to determine if biofilm transport channels are conserved across diverse phylotypes and to better understand their potential role in polymicrobial biofilm physiology.



## P6.14 - PSEUDOMONAS AERUGINOSA DRIVES GROWTH AND BIOFILM FORMATION IN OBLIGATE ANAEROBES FROM CHRONIC WOUND BIOFILMS

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**Background & Aims:** Chronic wound biofilms comprise diverse aerobic and anaerobic microorganisms associated with impaired healing. Gram-positive anaerobic cocci (GPAC) frequently dominate non-healing wounds, coexisting with facultative pathogens, yet their interspecies interactions remain poorly understood. We systematically investigated mixed-species biofilm interactions among dominant diabetic foot ulcer (DFU) isolates to determine how metabolic interactions shape biofilm formation in these communities.

**Methods:** Eight clinical DFU wound isolates, selected based on their high prevalence and recurrent co-occurrence identified in our preliminary systematic DFU microbiota analyses, were screened for biofilm formation and assembled into all four-species communities (n = 70). Biofilms were grown anaerobically in Wilkins-Chalgren medium (72 h, 37°C), and biomass quantified by crystal violet staining relative to monoculture-derived expectations to identify interaction effects. The combination showing the highest synergistic effect was selected for further analysis. Biomass and CFU were quantified in pairwise to four-species biofilms, with separate enumeration of *Pseudomonas aeruginosa* and pooled obligate anaerobes. Cell-free supernatants (CFS) derived from 24-, 48-, and 72-h *P. aeruginosa* monocultures were tested for effects on biofilm formation by obligate anaerobes.

**Results:** Systematic screening of mixed-species combinations revealed that only 9/70 combinations exhibited synergistic increases in biofilm biomass, whereas 55.1% resulted in significant reductions. The strongest effect was observed in a four-species consortium comprising *Anaerococcus vaginalis*, *Peptoniphilus harei*, *Finnegoldia magna*, and *P. aeruginosa*. Although these strains produced negligible biofilm in monoculture, the consortium generated a 7.5-fold increase in biomass relative to monoculture-derived expectations ( $P < 0.0001$ ). Pairwise co-culture with *P. aeruginosa* increased *A. vaginalis* and *F. magna* CFU by ~2.3-log and ~2.9-log, respectively ( $P < 0.05$ ) compared to their monoculture growth. Total obligate anaerobe counts showed a significant increase in parallel. *P. aeruginosa* CFUs remained unchanged across all combinations with obligate anaerobes. Moreover, CFS derived from 48-h *P. aeruginosa* monocultures enhanced biofilm formation in *P. harei* monocultures, *P. harei*-*A. vaginalis* co-cultures, and three-species obligate anaerobe communities by 2.4-, 2.8-, and 1.5-fold, respectively, relative to corresponding cultures without CFS ( $P < 0.05$ ).

**Conclusions:** Cooperative interactions between *P. aeruginosa* and obligate anaerobes within mixed-species chronic wound biofilms promote synergistic polymicrobial community assembly and provide a potential mechanistic explanation for anaerobe enrichment in non-healing wounds.



## P6.15 - PHOTODYNAMIC INACTIVATION AND FARNESOL AS POTENTIAL GAME CHANGERS IN THE ELIMINATION OF FRIGHTENING NOSOCOMIAL COMBINATION: CANDIDA ALBICANS AND ENTEROCOCCUS FAECIUM BIOFILMS

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**Background & Aims:** The effective treatment of persistent medical infections and diseases associated with repeated contamination by polymicrobial biofilms is essential but challenging. This issue is particularly concerning given the widespread prevalence of vancomycin-resistant enterococci (VRE) in hospitals worldwide. However, compared with *Enterococcus faecalis*, relatively little is known about the virulence and pathogenesis of *Enterococcus faecium*. As an alternative to conventional antimicrobial therapy, photodynamic inactivation (PDI) using Phloxine B and a green laser was evaluated for the eradication of mixed-species biofilms formed by *Candida albicans* and *E. faecium*. In addition, the natural fungal secondary metabolite farnesol was investigated as a quorum-sensing molecule and as a potential agent for biofilm inhibition.

**Methods:** Standard characterisation methods, including metabolic assay and crystal violet (CV) staining, were used to characterise single and dual-species biofilms formed by *C. albicans* (SC5314) and *E. faecium* (collection strain CCM7167 and VRE M17773). However, inoculum size and medium selection were also important steps. *Galleria mellonella* larvae were used to study immunity following post-infection. Viable plate counting served for assessing cell survival in mixed biofilms and after farnesol (0.1 and 1 mM) exposure, as well as for PDI (0.5 mM Phloxine B and laser  $\lambda = 538$  nm for 5 min). Expression of genes involved in biofilm development was investigated by real-time RT-qPCR, specifically the *BCR1*, *ALS3*, and *HWP1* genes for *C. albicans*.

**Results:** The most metabolically active biofilm was formed by the mixed SC5314 and CCM7167 strains, but CV staining showed no significant differences in the biomass of dual-species biofilms. Regarding adhesin expression, the most up-regulated gene was *BCR1* (biofilm and cell wall regulator) in 48-h matured biofilms. 1 mM farnesol eliminated *E. faecium* cells in both single and polymicrobial biofilms. PDI was shown to be effective using a non-toxic concentration of PhB, mainly against enterococcal strains. Genes encoding the peptides gallerimycin and galiomycin were up-regulated on the third day in all tested groups (mono- or dual- infection), and the survival rate of larvae fell to less than 50% for the VRE isolate.

**Conclusions:** Taken together, the results of this study indicate that biofilms formed by VRE can be effectively treated with PDI and farnesol.



## P6.16 - THE EFFECTS OF EPOXYTIGLIANE EBC-1013 ON OBLIGATE ANAEROBIC CHRONIC WOUND BACTERIA

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**Background & Aims:** Poor healing in chronic wounds such as venous leg ulcers (VLUs) and diabetic foot ulcers (DFUs) has long been attributed to bacterial biofilms in the wound bed, which are dominated by obligate and facultative anaerobes. The semi-synthetic epoxytigliane EBC-1013 has previously demonstrated strong antimicrobial activity including in biofilm disruption, membrane dysregulation and antimicrobial potentiation in oral and wound pathogens. However, the obligate anaerobic species prominent in VLUs and DFUs have yet to be evaluated. In this study, we sought to compare the antimicrobial activity of EBC-1013 against a range of obligate anaerobe and facultative anaerobe wound isolates.

**Methods:** Phenotypic proliferation assays were performed on clinical chronic wound isolates treated with EBC-1013 at minimum inhibitory concentration (MIC) or  $\frac{1}{2}$ MIC, alongside ethanol-vehicle and growth-medium controls (n = 3). Facultative anaerobes (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Corynebacterium striatum*, *Enterobacter hormaechei*, *Serratia marcescens*) were grown aerobically in Mueller–Hinton Broth (48 h, 37 °C). Obligate anaerobes (*Bacteroides fragilis*, *Prevotella bivia*, *Peptoniphilus asaccharolyticus*) and aerotolerant anaerobe *Streptococcus agalactiae* were grown anaerobically in Fastidious Anaerobe Broth (72 h, 37 °C). Membrane permeability assays were conducted on isolates treated with EBC-1013 (8–512 µg/mL) during exponential growth using SYTOX Green. Positive (70% isopropanol) and negative (vehicle) controls were included.

**Results:** Facultative anaerobes exhibited a high tolerance to EBC-1013, maintaining growth at concentrations >512 µg/ml, although with a modest increases in lag-phase compared to the untreated controls. In contrast, obligate and aerotolerant anaerobes demonstrated substantially greater sensitivity to EBC-1013, showing near-complete inhibition at 8–32 µg/ml. Permeability assays indicated minimal membrane disruption in Gram-negative facultative anaerobes, (up to 512 µg/ml;  $P>0.05$ ), whereas Gram-positive facultative anaerobes and (both Gram-positive and negative) obligate anaerobes showed significant increases in permeability at low EBC-1013 concentrations (typically 32–64 µg/ml).

**Conclusions:** This study demonstrates a clear oxygen-dependent difference in the antimicrobial susceptibility of chronic wound anaerobes to EBC-1013 and provides mechanistic evidence of selective membrane activity. This species-level information is clinically relevant for targeting the anaerobic component of chronic wounds and will guide future testing in polymicrobial in-vitro biofilm systems to better model clinical environments. EBC-1013 is currently undergoing evaluation in human phase I studies in chronic venous leg ulcers (ACTRN12624000544572).



## P6.18 - FUNGAL-BACTERIAL INTERACTIONS IN DUAL-SPECIES ORAL BIOFILMS FORMED BY CANDIDA ALBICANS AND AGGREGATIBACTER ACTINOMYCETEMCOMITANS

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**Background & Aims:** Polymicrobial biofilms play a key role in the pathogenesis of periodontitis and are associated with high rates of diagnostic and therapeutic failure, partly due to limited fundamental and translational understanding of fungal-bacterial interactions in oral biofilms. This study aims to investigate interaction dynamics within dual- species biofilms formed by *Candida albicans* and *Aggregatibacter actinomycetemcomitans*, two clinically relevant oral pathogens. Previous studies have highlighted the influence of environmental factors, such as carbon dioxide availability, and spatial relationships, including hyphae-associated attachment within the gingival sulcus.

**Methods:** Biofilm biomass in dual- versus mono-species biofilm was quantified using crystal violet assays. Biofilm cell viability was assessed by CFU enumeration on selective media under defined growth conditions (Sabouraud Dextrose Agar, 72 h at 37°C without 5% CO<sub>2</sub>; Blood Agar, 72 h at 37°C with 5% of CO<sub>2</sub>). Antimicrobial susceptibility to fluconazole and azithromycin was evaluated in single- and mixed-species planktonic cultures using clinically relevant concentrations for oral infections. Spatial organisation and coexistence of both microorganisms were examined by confocal laser scanning microscopy using PNA-FISH.

**Results:** Mixed-species biofilms demonstrated stable coexistence of both microorganisms without significant mutual inhibition. The presence of *C. albicans* resulted in a significant increase in *A. actinomycetemcomitans* abundance ( $\pm 2.8 \text{ Log}_{10}$ ,  $p < 0.05$ ), while *C. albicans* levels remained stable ( $\pm 0.3 \text{ Log}_{10}$ ,  $p > 0.05$ ). Total biofilm biomass was reduced in dual-species biofilms compared with mono-species biofilms, reflecting ecological adaptation to coexistence within a shared environment. The greater reduction observed in *C. albicans* biomass was not associated with loss of cellular viability, indicating stabilization of fungal survival under dual-species conditions. Antimicrobial susceptibility testing showed reduced susceptibility of both microorganisms to fluconazole and azithromycin in planktonic culture. Confocal microscopy revealed structural coexistence without evidence of competitive exclusion. Notably, *C. albicans* hyphae served as a scaffold for *A. actinomycetemcomitans* attachment.

**Conclusions:** Dual-species biofilm formation between *C. albicans* and *A. actinomycetemcomitans* results in a non-reciprocal synergism that promotes bacterial persistence while maintaining fungal survival. Polymicrobial organization confers decreased susceptibility to standard antifungal and antibacterial agents. These findings highlight the importance of considering microbial interactions when designing therapeutic strategies for polymicrobial oral infections.



## P6.19 - IN VITRO EVALUATION OF POLYMICROBIAL BIOFILMS OF STAPHYLOCOCCUS AUREUS AND CLINICALLY RELEVANT CANDIDA SPP FOR BIOCIDAL ACTIVITY STUDIES.

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**Background & Aims:** Polymicrobial biofilms of *Staphylococcus aureus* and *Candida* spp. represent a major challenge in chronic and device associated infections due to the increase of antimicrobial-resistance pathogens. Bacterial-fungal interactions within biofilms can significantly influence biomass production, microbial viability, and tolerance to antimicrobial treatments. This study aimed to optimize a reproducible *in vitro* model of mono and polymicrobial biofilms and to evaluate their response to a dendritic system and a natural compound derived from *Lonicera japonica*.

**Methods:** Biofilms were developed in 96 well microtiter plates using standardized inocula of *S. aureus* and five clinically relevant fungal species: *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Nakaseomyces glabrata* (formerly *Candida glabrata*), and *Candidozyma auris* (formerly *Candida auris*). Polymicrobial combinations included clinically relevant mixtures. Total biomass was quantified by crystal violet staining measured at 630 nm. Biofilms were categorized as weak, moderate, or strong according to Optical Density Cutoff (ODc) criteria. Microbial viability and response to the dendrimer and *Lonicera japonica* compound were assessed using a metabolic assay (resazurin) and viable cells were quantified by Drop Plate on selective media (Plate Count Agar for bacteria and Sabouraud agar for fungi). Experiments were performed in triplicate. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration/Minimum Fungicidal Concentration (MBC/MFC) values were determined following standard procedures.

**Results:** The model produced reproducible mono and polymicrobial biofilms. *C. tropicalis* exhibited higher biofilm biomass and Colony Forming Units (CFU) counts, indicating enhanced growth ( $p < 0.05$ ). Dual species models were analyzed in detail, showing that co-culture with *S. aureus* modified biofilm density and viability. ODc classification distinguished weak, moderate, and strong biofilms, while quantitative CFU analysis allowed comparison of relative growth. Drop Plate assays confirmed viability of both bacterial and fungal populations, and MIC/MBC/MFC determination demonstrated the model's suitability for assessing antimicrobial susceptibility in complex biofilms.

**Conclusions:** This optimized *in vitro* model provides a robust and reproducible platform for studying clinically relevant polymicrobial biofilms and bacterial fungal interactions. The combined use of ODc classification and CFU quantification allows detailed assessment of biofilm density and antimicrobial susceptibility, supporting the development of novel therapeutic strategies against chronic and device associated infections.



## P8.1 - IDENTIFYING BIOFILM-FORMING CONDITIONS IN PROBIOTIC BACTERIA

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**Background & Aims:** Probiotics are defined as “bacteria which, when administered in an effective dose, confer a benefit on the health of the patient”. Probiotics have been proven to assist with the treatment of conditions such as ulcerative colitis, inflammatory bowel disease, hypercholesterolaemia, antibiotic-associated diarrhoea, and various allergic diseases. Whilst there are many examples of probiotic products available on the market, some of the most popular with consumers are yoghurt drinks. The probiotics market is worth an estimated \$15 billion per year and is growing by 7% per year. For greatest effectiveness of the resultant product, it is crucial that maximal bacterial survival is achieved; this is important both during extended shelf storage, and also to maximise the concentration of bacteria that are able to survive and proliferate in the human gut. Multiple novel approaches have been explored for the development of systems to enhance the survival of probiotic bacteria in formulated products. Previous studies have suggested that biofilm formation may enhance probiotic survival. As such, in this project, we are investigating culture conditions that lead to increased probiotic biofilm production and utilising this information to inform the development of a novel system for probiotic bacteria formulation in the dairy industry.

**Methods:** Using standardised assays, we have investigated the effects of acidic pH, increased osmolarity, and an altered carbon source on probiotic biofilm formation, cell surface hydrophobicity, and growth kinetics.

**Results:** The aforementioned conditions had significant effects on all three conditions. Increasingly acidic pH was detrimental to both biofilm formation and growth, whilst the effects of osmolarity and altered carbon source were less easily defined.

**Conclusions:** These experimental findings provide valuable insight to how these probiotic strains interact with their environment, and are foundational to our future work in developing novel strategies for delivering probiotics to consumers.



## P10.1 - ENHANCING BACTERIOPHAGE ACTIVITY AGAINST PSEUDOMONAS AERUGINOSA BIOFILMS FORMED BY CLINICAL ISOLATES THROUGH DIRECTED EVOLUTION

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**Background & Aims:** Biofilms formed by *Pseudomonas aeruginosa* are highly tolerant to conventional antimicrobials, leading to persistent infections and treatment challenges. Given the limited efficacy of existing control methods, developing novel strategies to combat biofilms remains a critical research focus. Bacteriophages, viruses that specifically infect bacteria, offer a targeted therapeutic approach to biofilm-related infections. However, their efficacy against clinical biofilms remains poorly understood. This study evaluated the biofilm-control potential of phages against clinical *P. aeruginosa* isolates and explored enhancement of phage activity through directed adaptation to biofilms.

**Methods:** Twenty-four-hour biofilms of eight clinical *P. aeruginosa* isolates were treated with four bacteriophages at two concentrations ( $10^7$  and  $10^8$  PFU/mL) and two exposure times (6 and 24 h). Biofilm reduction was quantified using colony-forming unit (CFU) counts and resazurin-based metabolic activity assays. The least effective phage (vB\_Pae-TbilisiM32) was further analyzed using confocal microscopy and subjected to directed adaptation over 8 days to improve biofilm-control efficacy.

**Results:** All clinical isolates formed dense 24-h biofilms ( $\approx 2 \times 10^8$  CFU/mL), with CFU counts correlating with metabolic activity. Across all bacteria-phage combinations, higher phage concentrations produced greater biofilm reduction, with maximal effect at 6 h post-treatment (up to 2-log CFU reduction; 41% metabolic activity reduction) followed by partial regrowth at 24 h (up to 1-log CFU reduction; 15.4% metabolic activity reduction). Confocal imaging confirmed transient reduction in biofilm thickness at 6 h, with recovery to untreated levels by 24 h. Directed adaptation of vB\_Pae-TbilisiM32 enhanced biofilm reduction compared to the wildtype phage across all 5 adapted variants, currently under genomic and activity characterization.

**Conclusions:** Bacteriophages can rapidly reduce *P. aeruginosa* biofilms, but single phage applications are limited by population regrowth, emphasizing the need for combination strategies and pre-treatment biofilm testing due to phage-bacteria specificity. Directed phage adaptation to biofilms is a promising approach to improve phage efficacy and may support more effective biofilm-targeted therapies. This study was conducted within the framework of the European Union-funded project ASCLEPIUS.



## P10.2 - NOVEL LYTIC BACTERIOPHAGES FOR COMBATting BIOFILM- ASSOCIATED INFECTIONS BY PSEUDOMONAS AERUGINOSA AND STAPHYLOCOCCUS SPP

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**Background & Aims:** Biofilm-associated infections caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus* are difficult to treat due to their increased tolerance to antimicrobials. Bacteriophages offer a promising alternative, but their efficacy against clinically relevant, biofilm-forming strains remains underexplored. Understanding strain-level heterogeneity in biofilm formation and phage susceptibility is therefore critical for rational phage therapy development. In this study, we aimed to characterize the biofilm-forming capacity, genetic diversity, and phage susceptibility of clinical respiratory isolates, and to identify strain-phage combinations suitable for potential therapeutic application.

**Methods:** A total of 106 *P. aeruginosa* and 83 *Staphylococcus* isolates were recovered from sputum, nasal, and pharyngeal samples from the Eliava Institute and were screened for phage susceptibility using monophages and phage lysates. Biofilm formation was assessed using crystal violet staining followed by quantitative colony-forming unit (CFU) enumeration. ERIC PCR was performed to assess genomic diversity of biofilm- producing strains, revealing wide variability among isolates and confirming that the strain panel represented a heterogeneous clinical population.

**Results:** ERIC PCR analysis revealed substantial genomic diversity among *Pseudomonas aeruginosa* biofilm producing strains. Among *P. aeruginosa* isolates, 85% showed susceptibility to at least one phage, with phage lysates demonstrating higher overall activity than monophages. Biofilm screening identified 30 out of 40 tested *P. aeruginosa* strains as biofilm producers, with biofilm densities reaching up to  $10^{10}$  CFU/ml. In *Staphylococcus* spp., only 4% of strains were fully phage-resistant, and phage SB-1 showed the broadest activity, lysing 91% of tested strains. Biofilm formation among *Staphylococcus* strains was moderate but reproducible. Eight novel monophages active against biofilm-forming *P. aeruginosa* were isolated.

**Conclusions:** This study establishes a well-characterized panel of biofilm-forming clinical isolates and corresponding lytic bacteriophages, providing a robust foundation for future phage-biofilm interaction studies and therapeutic development.

The research was carried out in the framework of the ASCLEPIUS project.



## P10.3 - TRANSIENT ANTIBIOTIC EFFICACY AGAINST ENTEROCOCCUS FAECALIS BIOFILMS IN AN IN VITRO VASCULAR GRAFT INFECTION MODEL

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**Background & Aims:** Enterococcus faecalis represents an important species recovered from clinical cases of Vascular Graft and Endograft Infections (VGEI). E. faecalis is difficult to eradicate due to its in-trinsic antibiotic resistance and the ability to form antibiotic tolerant biofilms on vascular pro-s-theses forming VGEI. Biofilm embedded bacteria can survive antibiotic concentrations far exceeding standard therapeutic levels. To evaluate on antibiotic efficacy against E. faecalis biofilms in an in vitro model of Vascular graft and Endograft infections.

**Methods:** The previously established Staphylococcus aureus VGEI model was further evolved to use E. faecalis. Endovascular prosthesis segments were inoculated with the E. faecalis clinical isolate 39898 and incubated for 24 hours to allow biofilm formation. Following incubation, the segments were exposed to 24 hours of antibiotic treatment with ampicillin, moxifloxacin, and tobramycin at concentrations corresponding to 1×MIC, 4×MIC, and 16×MIC for each anti-bi-otic, based on the initial MIC determination. Treatment efficacy was assessed by quantitative bacteriology of the prostheses after by sonication, serially diluted, and plated to determine CFU/mL. In addition, extent of regrowth from treated prosthesis was evaluated by means of the certika method.

**Results:** At 1× MIC and 4x MIC, none of the antibiotics had any significant effect on the biofilms. At 16× MIC, two of the three agents exhibited marked activity against the biofilm derived cells. Tobramycin had a ~4 log reduction ( $p<0.0001$ ). Ampicillin showed a ~5.3 log reduction ( $p<0.001$ ). In contrast, moxifloxacin showed no significant reduction. Using the Certika method to evaluate regrowth from prostheses, antibiotic exposure produced dose dependent inhibition. At 4× MIC, ampicillin caused a 6 hour delay of regrowth, moxiflox-acin produced a 5 hour delay, and tobramycin resulted in a 2 hour delay relative to controls. At 16× MIC, ampicillin produced a 13 hour delay of regrowth. Moxifloxacin delayed regrowth for 5 hours, and tobramycin for 8 hours before regrowth resumed.

**Conclusions:** Collectively, these findings demonstrate pronounced biofilm tolerance in E. faecalis, as well as agent- and concentration-dependent responses using a representative VGEI model. Com-plete biofilm eradication was not achieved, and regrowth occurred within 24h following com-pletion of treatment, underlining the need for effective adjunctive strategies.



## P10.4 - 5-FLUORO-CYTOSINE: AN ANTIMETABOLITE WITH ANTIBIOFILM PROPERTY AGAINST UROPATHOGENIC ESCHERICHIA COLI STRAINS

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**Background & Aims:** Uropathogenic *Escherichia coli* (UPEC) is the most frequent type of *E. coli* that can infect tissues outside the intestines and is commonly associated with urinary tract infections (UTI). UTI treatment is often complicated by antibiotic resistance and UPEC ability to survive as persisters and in biofilms, leading to chronic, recurrent infections. Alternatives to antibiotics have been proposed to overcome this limitation, e.g., antivirulence strategies that aim to reduce virulence, thereby preventing resistance emergence. For example, 5-fluoro- cytosine (5-FC) showed novel antivirulence and antibiofilm activity against UPEC at subinhibitory concentrations and restored antibiotic susceptibility of the biofilm to beta-lactams. The aim of this work was to explore the molecular mechanisms behind 5-FC activity against UPEC strains.

**Methods:** The laboratory strain *E. coli* MG1655 and the UPEC *E. coli* EC73 isolate from chronic prostatitis were studied. Phenotypic assays evaluating adhesion (crystal violet (CV) and Congo red (CR) assays), MIC, and yeast aggregation were performed at increasing concentrations of 5-FC. 5-FC effect on virulence-related genes and global regulators expression was evaluated by RT-qPCR. MG1655 samples were collected at multiple time points within 2 hours post-treatment; for the EC73 strain, analysis was performed at different growth phases. Antibiotics persistence to beta-lactams and fluoroquinolones was evaluated at different concentrations of 5-FC.

**Results:** Antibiofilm property was confirmed for both strains in CV and CR assays, while 5-FC treatment didn't show any effect on aggregation. At the transcriptional level, 5-FC effect on the *E. coli* MG1655 was observed at early time points, resulting in increasing global regulators responding to nucleotide levels (Fis), pyrimidine biosynthetic genes (CarA), and to cell-wall stress (wza), leading to a quick inhibition of downstream virulence targets and curli fibers. On *E. coli* EC73, the effect seemed milder, with adhesion-related genes mostly affected. The emergence of persisters was reduced by 5-FC treatment exclusively when beta-lactams were used.

**Conclusions:** Our data confirm the 5-FC antivirulence properties across different *E. coli* strains, showing a rapid transcriptional effect that acts through the global regulator Fis and via the cell-wall stress response. In addition, we highlighted a previously unknown effect that reduces antibiotic persisters, revealing another clinically relevant property of the drug.



## P10.5 - GLYCOPEPTIDE AND LIPOGLYCOPEPTIDE-MITOMYCIN C CONJUGATES FOR TARGETED DELIVERY TO BIOFILM INFECTIONS

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**Background & Aims:** Biofilm infections persist due to antibiotic tolerance of non-growing persister cells. The antineoplastic mitomycin C is known to be effective against persister cells, but it is also cytotoxic and immunosuppressive. To increase therapeutic impact and minimize toxicity, we developed mitomycin C conjugates with a thiol-reactive linker to a targeting moiety. Vancomycin, teicoplanin, and dalbavancin were used as targeting moieties, enabling broad-spectrum targeting of Gram-positive pathogens.

**Methods:** Various strains of *S. aureus*, *S. epidermidis*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *A. baumannii*, and *K. oxytoca* were treated with free mitomycin C, (lipo)glycopeptide-mitomycin C conjugates, or the admixture of (lipo)glycopeptides and mitomycin C for 15 minutes under non-growing conditions followed by a washing step to remove unbound drug, and 24 h incubation in buffer with or without N-acetylcysteine (NAC) to trigger drug release from the conjugate. Antimicrobial efficacy was quantified by CFU enumeration. Fluorescently labelled vancomycin, teicoplanin or dalbavancin were used to document targeting capability.

**Results:** CLSM imaging showed that lipoglycopeptides targeted bacteria more effectively compared with glycopeptides. The fluorescence signal from surface-bound teicoplanin and dalbavancin was much higher than vancomycin, and the drugs even bound to stationary-phase cultures, vancomycin-resistant strains, and biofilms. Lipoglycopeptide-conjugated mitomycin C exhibited significant antimicrobial activity against a wide range of Gram-positive pathogens after only 15 min exposure. The activity was comparable to the impact of 24 h exposure free mitomycin C. When tested against biofilms, teicoplanin conjugates displayed higher antimicrobial activity than dalbavancin or vancomycin conjugates.

**Conclusions:** We demonstrate targeted delivery of antineoplastic prodrug using glycopeptide and lipoglycopeptide antibiotics as the targeting agents. The efficacy of this therapeutic against non-growing bacteria, resistant strains, and biofilms suggests that this type of drug conjugate offers a new strategy for treating chronic biofilm infections.



## P10.6 - A PROMISING FLAVONOID SCAFFOLD FOR ANTI-BIOFILM AGENT DEVELOPMENT

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**Background & Aims:** Biofilm-associated infections represent a major clinical challenge due to their intrinsic tolerance to antimicrobial therapy and their contribution to persistent and recurrent infections. The urgent need for effective anti-biofilm strategies has driven the search for novel compounds with enhanced biological activity. In this study, we investigated the anti-biofilm potential of a synthetic sulfur-containing tricyclic flavonoid (BrCl- flav) against *Candida* biofilms.

**Methods:** The effect of BrCl-flav on different stages of biofilm development (initial adhesion, biofilm formation, and mature biofilms) was evaluated using crystal violet staining and MTT metabolic activity assay. Structural alterations in mature biofilms were further analyzed by confocal laser scanning microscopy (CLSM).

**Results:** BrCl-flav significantly inhibited early biofilm development, affecting both bacterial adhesion and biofilm formation. Biofilm biomass was reduced by 90% at 15.62 µg/mL (equivalent to MIC). The compound also showed notable activity against mature biofilms, leading to a reduction of approximately 90% at 62.5 µg/mL. CLSM analysis revealed marked alterations in biofilm architecture following treatment. Moreover, metabolic activity of biofilm-embedded cells decreased by 58% compared to untreated controls at 7.8 µg/mL, indicating substantial impairment of biofilm viability.

**Conclusions:** BrCl-flav exhibited significant inhibitory effects across all stages of biofilm development, including mature biofilms. These findings highlight its potential as a promising scaffold for the development of novel anti-biofilm agents targeting resistant microbial infections.



## P10.7 - THE ROLE OF ANTIMICROBIAL EFFLUX IN SHAPING BIOFILM SPATIAL STRUCTURE

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**Background & Aims:** Biofilms are multicellular assemblages of bacteria living in a self-produced extracellular matrix. Different mechanisms, including the development of highly tolerant persister cells, increased efflux pump expression and biofilm structure make them difficult to kill. Efflux pumps extrude various antimicrobials out of the cells. AcrAB-TolC is the major efflux pump in *E. coli*. It has been shown that efflux pumps can be linked to cell-to-cell-interactions, membrane integrity, and by global regulators to persister and biofilm formation. This study investigates the effect of efflux-mediated spatial interactions in shaping biofilm structure.

**Methods:** To this end, we generated biofilm-forming, fluorescently labeled *E. coli* strains (AR3110) with a knockout of *acrB* or with *acrAB* under the control of an inducible promoter, leading to different levels of efflux activity. The strains were characterized in terms of their antimicrobial susceptibility and efflux activity. To investigate the spatial arrangement of strains with different efflux activity in a colony biofilm, strains labeled with different fluorescent proteins and efflux activity were mixed and grown on agar supplemented with antimicrobials at sub-inhibitory concentrations.

**Results:** Analysis of colony biofilms with fluorescence microscopy showed that the absence of the AcrAB-TolC efflux pump affects the structure of sector formation and morphology within the colony depending on the supplemented antimicrobial substance and its concentration. We observed relatively large sectors with similar surface area for high efflux and low efflux cells in the absence of antimicrobials and at low concentrations. Higher ampicillin and benzalkonium chloride concentrations lead to reduced presence of efflux deficient cells. In contrast, sectors disappeared in the presence of the low efflux strain with increasing concentration upon exposure to tetracycline. Even though kanamycin is not a substrate of AcrAB-TolC, a strong effect on the spatial arrangement in the biofilm structure and apparent mutants were observed. Ongoing experiments on the single-cell level and mathematical modelling shed light on the mechanisms that underlie efflux-mediated spatial effects in biofilms.

**Conclusions:** Taken together, our data suggest that efflux shapes cell-to-cell interactions and these interactions affect the spatial arrangement of cells with different efflux activity in biofilms.



## P10.8 - WHEN CLEANING ISN'T ENOUGH: SUPERIOR ANTI-BIOFILM ACTIVITY OF DISINFECTANTS OVER DETERGENTS AGAINST MDR CLINICAL ISOLATES

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**Background & Aims:** The presence of multidrug-resistant Gram-negative (MDR) bacteria—particularly when organized in biofilms within the patient environment—poses a growing challenge for hospital hygiene. However, most outbreak investigations do not evaluate biofilms of multidrug-resistant bacteria against disinfectants or surface cleaners, leaving the effectiveness of these agents insufficiently proven.

**Methods:** We evaluated four surface disinfectants and two surface cleaners against biofilms of carbapenemase-producing (CPE) bacteria. The disinfectants included alcohol-, QUAT-, and glutaraldehyde-QUAT-based products, as well as potassium monopersulfate; the cleaners were alcohol/glycol- or probiotic-based. Biofilms were formed from three CPE *Citrobacter freundii* (CPE-CF) and three CPE *Pseudomonas aeruginosa* (CPE-PA) strains. A susceptible *C. freundii* strain and a susceptible *P. aeruginosa* reference strain (DSM 22644) served as controls. The CPE strains originated from patients and sinks involved in an outbreak at the Vienna General Hospital, an academic tertiary care hospital, and were characterized by next-generation sequencing. Experiments followed ASTM E2799-17 using viable colony counts (VCC). Biofilm biomass was measured using crystal violet staining, and metabolic activity using the XTT assay. Biofilms grown at 25 °C and 37 °C were analyzed after 24 hours of exposure. All tests were performed in triplicate.

**Results:** Disinfectants reduced viable bacteria in biofilms by  $>5 \log_{10}$ , except QUATS, which only inhibited the control strains. At 25 °C, 24-h CPE biofilms showed higher biomass than at 37 °C (OD 0.1771 vs. 0.1321 for CPE-PA; 0.1410 vs. 0.0623 for CPE-CF). At 25 °C, the glutaraldehyde/QUATS disinfectant increased CPE biofilm biomass (CPE-PA: 0.1675 vs. 0.1430; CPE-CF: 0.1390 vs. 0.1180). Disinfectants reduced the metabolic (XTT) activity of CPE biofilms by 53% and 43%, comparable to reductions observed in control strains (56% and 45%). CPE biofilm metabolic activity increased from 50% at 4 h to 60% at 24 h, suggesting partial recovery.

**Conclusions:** Surface disinfectants demonstrated robust anti-biofilm efficacy against MDR CPE isolates, whereas cleaners showed insufficient activity. Effective outbreak control requires disinfectant-based strategies rather than reliance on cleaning alone, particularly in environments contaminated with biofilm-forming MDR Gram-negative pathogens.



## P10.9 - MODULATION OF THE AGR QUORUM SENSING SYSTEM BY LIGHT-ACTIVATED NANOCOMPOSITE WITH ERYTHROSINE B IN STAPHYLOCOCCUS AUREUS

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**Background & Aims:** The accessory gene regulator (*agr*) quorum sensing (QS) system of *Staphylococcus aureus* participates significantly in regulating virulence and biofilm development, both through its activation and inhibition. This study aimed to assess the effect of photoactive nanomaterials functionalized with erythrosine B (EryB) on the modulation of the *agr* QS system in methicillin-resistant *S. aureus* (MRSA) strains. In particular, the question was whether photodynamic inactivation (PDI) mediated by an EryB-based nanocomposite affects *agr* activity and, consequently, whether it would influence biofilm development in clinical MRSA isolates with varying *agr* functionality.

**Methods:** The activity of the *agr* system was evaluated using the CAMP test and quantitative PCR (qPCR) analysis of the *hld* gene expression, which is part of RNAIII that encodes  $\delta$ -hemolysin. Biofilm formation was analysed using the crystal violet assay and fluorescence microscopy. Antibiofilm efficacy was assessed by determining colony-forming units (CFU).

**Results:** The CAMP test identified MRSA strains S66 and S68 as *agr*-positive, while MRSA strain S73 was *agr* -negative. In these strains, the biofilm was less compact compared to the dense biofilm formed by the *agr* -negative strain. The anti-biofilm effectiveness on the nanocomposite with EryB after irradiation reduced the growth of biofilm cells by 100- to 1000-fold compared to the biofilm on polyurethane alone. The qPCR analysis showed a significant decrease in relative *hld* gene expression in *agr*-positive strains after irradiation compared to non-irradiated samples, confirming that PDI suppresses *agr* activity. Importantly, despite the decrease in *agr* expression, no increase in biofilm formation was observed. On the contrary, biofilm viability was significantly reduced in both *agr*-positive and *agr*-negative strains, indicating that the anti-biofilm effect of the EryB-based nanocomposite occurs independently of the functional state of the *agr* QS system.

**Conclusions:** The photoactive nanocomposites functionalized with EryB effectively inhibit MRSA biofilm formation, regardless of the state of the *agr* QS system, and are a promising platform for further research, especially in the healthcare sector.

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## **P10.10 - REGULATORY ROLES OF NOVEL SMALL NON-CODING RNAS IN BIOFILM FORMATION, AND ANTIMICROBIAL RESISTANCE IN STRONG BIOFILM-FORMING ACINETOBACTER BAUMANNII CLINICAL ISOLATE**

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**Background & Aims:** *Acinetobacter baumannii* is a multidrug-resistant nosocomial-pathogen of critical priority for new antibiotic research. Its ability to form robust biofilm on host tissues and abiotic surfaces exacerbates the critical concerns regarding antimicrobial resistance. Small RNAs (sRNAs) regulate genes involved in biofilm formation, antimicrobial resistance, and virulence of various bacterial species including *A. baumannii*. In the current study, a transcriptomic analysis of the strong biofilm-forming, carbapenem-susceptible *A. baumannii* ST10 (IC8) was conducted to understand the underlying molecular mechanisms of strong biofilm formation and decreased antimicrobial susceptibility in biofilm conditions.

**Methods:** In our lab, 225 *A. baumannii* isolates were assessed for biofilm-forming ability using the Calgary Biofilm Device (CBD) and antimicrobial susceptibility to a panel of antimicrobial agents including carbapenems. Among clinical isolates originally derived from human subjects, *A. baumannii* ST10 (IC8) exhibited the strongest biofilm formation, broad planktonic antimicrobial susceptibility, and reduced carbapenem susceptibility in biofilm. Transcriptome profiling was performed on total RNA samples extracted from the planktonic and biofilm cells in three independent replicates. Using standard bioinformatic analyses, sRNAs were identified from intergenic regions. The secondary structures and potential targets of these sRNAs were predicted using RNAfold and IntaRNA, respectively. The differentially expressed sRNAs and other genes were verified using RT-qPCR.

**Results:** 1164 genes were differentially expressed, including four novel sRNAs. Notably, sRNA00007 and sRNA00048 were significantly upregulated in biofilm cells. In silico prediction showed that these sRNAs targeted a number of biofilm-associated genes: GABA transaminase, LysE translocator, and AraC family transcriptional regulator were downregulated, while TonB-dependent receptor, ExeM/NucH family extracellular endonuclease, and GGDEF domain-containing protein were upregulated in biofilm cells.

**Conclusions:** These and other differentially expressed genes may enhance biofilm formation, contribute to bacterial survival under stress, and promote antimicrobial resistance. The regulatory roles of sRNA00007 and sRNA00048 in biofilm formation, antimicrobial resistance, survival and stress response in *A. baumannii* will be further investigated using MS2 Affinity Purification coupled with RNA sequencing (MAPS).



## P10.11 - EVALUATION OF THE ANTIFUNGAL ACTIVITY OF DENDRITIC SYSTEMS AGAINST CANDIDA TROPICALIS BIOFILMS

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**Background & Aims:** *Candida tropicalis* causes invasive infections that are difficult to treat, especially in immuno-compromised patients or those who have undergone chemotherapy or broad-spectrum antibiotic treatments. These infections are associated with high mortality rates and are commonly related to resistance to azoles or echinocandins. The incidence of this type of infection continues to rise, making the search for new drugs of vital importance. Among these, dendritic systems stand out, which are the focus of this study. In this work, three dendritic systems (BDSQ024, BD132, and BDNG001) have been tested as antifungal agents against *C. tropicalis*.

**Methods:** Antifungal activity studies were carried out against planktonic cells, evaluating the inhibition of the biofilm formation and the eradication of preformed biofilms. Later, the cytotoxicity of the compounds was analyzed in HeLa cells. Based on these results, the most promising dendrimer was selected. Additional assays were then performed to better understand its mechanism of action, including the evaluation of morphological alterations by Scanning Electron Microscopy (SEM) and the analysis of reactive oxygen species production using confocal microscopy.

**Results:** The results identified BDNG001 as the most promising dendrimer, as it exhibited no cytotoxicity at any of the concentrations required to inhibit *C. tropicalis* biofilms. SEM analysis revealed swollen cells and a reduction in hyphal formation following treatment with the dendrimer. Additionally, confocal microscopy assays demonstrated that the treatment increased ROS production in *Candida* cells.

**Conclusions:** The results indicate that the dendrimer BDNG001 has strong potential as an antifungal agent against *Candida*. However, further studies are required to better understand its mechanism of action and to evaluate its toxicity in animal models.



## P10.12 - IN VITRO ACTIVITY OF DENTRITIC SYSTEMS AGAINST MRSA AND MSSA BIOFILMS

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**Background & Aims:** The search for new molecules effective against biofilms is crucial. In this regard, newly synthesized dendritic systems, such as dendrimers and dendrons, have become a promising alternative due to their versatility and low toxicity. The aim of this study was to determine the antibacterial activity of a dendritic system against two strains of *Staphylococcus aureus* (MSSA, 240 CECT and *S. aureus* resistant to ampicillin, 4004 CECT).

**Methods:** The activity of the compound was evaluated *in vitro* against planktonic cells, inhibiting biofilm formation, and eradicating established biofilms.

**Results:** The results obtained were promising in the biofilm and planktonic stages. While both strains were sensitive to the compound, the MIC and MBC were slightly lower for 4004 CECT. Regarding the treatment of forming biofilms, both 4004 CECT and 240 CECT showed sensitivity, reaching a MBC-B (Minimum Bactericidal Concentration in the Biofilm) of 4 mg/L. Finally, the *in vitro* assays against established biofilms showed the same behavior as observed in the planktonic stage: greater activity against 4004 CECT (Minimum Biofilm Eradicating Concentration, MBEC: 32 mg/L) than against 240 CECT (MBEC: 64 mg/L).

**Conclusions:** In conclusion, our compound demonstrates successful efficacy against both strains of *S. aureus* and may represent a promising candidate for the treatment of infections caused by this microorganism, even under biofilm-forming conditions.



## P10.13 - ENERGY, EFFLUX AND RESISTANCE IN BACTERIA

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**Background & Aims:** Efflux pumps are membrane proteins found in both Gram-negative and Gram-positive bacteria and are well known contributors of intrinsic and extrinsic antimicrobial resistance; however, studies have shown that these efflux pumps also play different roles in various aspects of biofilm formation and preservation. The contribution of efflux pumps to biofilm is by efflux of quorum sensing signals and metabolites, expulsion of toxic substances, indirectly influencing the regulation of biofilm associated genes and in the initial attachment stages of biofilm formation. This is further strengthened by the fact that deletion of efflux pumps has been shown to significantly decrease biofilm formation in various bacterial species (Baugh et al., 2012), (Bay et al., 2017). Whilst existing literature has shown that membrane potential is important for efflux activity, there remains a notable gap in how efflux and the redox state of the cell contribute to these behaviours and the underlying mechanisms by which this occurs is unclear. Furthermore, our understanding of how this link effects biofilm formation is limited. We aim to understand the relationship between efflux, energy and biofilm formation in *Salmonella enterica* serovar Typhimurium.

**Methods:** To investigate this, genetic and phenotypic methods were used to understand how altering the redox state of bacterial cells in a wildtype and efflux mutant would impact biofilm formation. Bacteria were modified with plasmids expressing either formate dehydrogenase (FDH1) of *Candida boidinii*, which reduces NAD<sup>+</sup> to NADH or NADH oxidase (NOX) of *Streptococcus pneumoniae*, which oxidizes NADH to NAD<sup>+</sup> (Arce- Rodríguez et al., 2022). The effect of altering NADH:NAD<sup>+</sup> ratio on biofilm formation was quantified using crystal violet assays and motility was measured using soft agar assays.

**Results:** Changes in the NADH:NAD<sup>+</sup> ratio resulted in the perturbation of biofilm formation in both cells harbouring the plasmids. Motility experiments using FDH1- and NOX-expressing cells showed that changing the NADH:NAD<sup>+</sup> ratio of bacteria reduced motility in comparison to the wild type strain.

**Conclusions:** These results demonstrate how the energy state of the cell is linked to biofilm formation in *Salmonella*; this link could provide new approaches to inhibit biofilm formation.

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## P10.14 - CANDIDA GLABRATA BIOFILMS UNDER VAGINAL CONDITIONS: IMPACT OF PH AND CORRELATION WITH ANTIFUNGAL RESISTANCE

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**Background & Aims:** Vulvovaginal candidiasis (VVC) is a common infection affecting women worldwide, caused by *Candida* species. Among these, *Candida glabrata* has become increasingly problematic due to its rising prevalence, reduced sensitivity to first-line antifungal treatments and capacity to develop resistance. This species also exhibits notable virulence factors, such as the ability to form biofilms, which hinder treatment effectiveness and increase the likelihood of recurring infections. Understanding how *C. glabrata* adapts and survives in the vaginal environment is essential for improving management strategies. This study aimed to explore how biofilm production relates to the vaginal pH and antifungal susceptibility.

**Methods:** This research focused on analyzing the biofilm-forming ability of 37 vaginal *C. glabrata* strains previously isolated from women with VVC. Biofilm formation was assessed in synthetic vaginal fluid at two vaginal pH levels: pH 4 (normal acidic pH) and pH 7 (elevated pH). Susceptibility to antifungal agents (fluconazole, ketoconazole and caspofungin) was evaluated using E-test method. Data on patient risk factors, such as prior infections, menopause, and other health conditions, were also collected via questionnaire.

**Results:** Results demonstrated that all strains could produce biofilms, though the amount of biomass varied among isolates. Interestingly, biofilm formation was significantly more robust at the normal vaginal pH of 4 for all strains, with this effect intensifying as biofilm biomass increased. This suggests that the acidic environment of the vagina may enhance certain virulence traits of *C. glabrata*. Regarding antifungal resistance, six strains were resistant to fluconazole, while 25 showed resistance to at least one antifungal drug. A potential link between high biofilm production and fluconazole resistance was observed, implying that resistant strains may be more prone to forming biofilms. Moreover, factors such as previous infections, menopausal status, and other health conditions appeared to contribute to antifungal resistance.

**Conclusions:** This study enhances our understanding of *C. glabrata*'s virulence mechanisms within the vaginal environment and highlights the importance of considering biofilm formation and resistance patterns when managing complicated VVC cases. Such insights are vital for developing more effective treatment strategies and reducing recurrence rates.



## P10.15 - COLD PLASMA OPENS ANTIBIOTIC SUSCEPTIBILITY WINDOWS IN IMPLANT-ASSOCIATED STAPHYLOCOCCUS AUREUS BIOFILMS

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**Background & Aims:** Orthopaedic implant infections are sustained by *Staphylococcus aureus* biofilms that tolerate antibiotic treatment despite retained susceptibility in standard planktonic assays. Cold atmospheric plasma (CAP) can enhance antimicrobial activity against biofilms, but it remains unclear whether this reflects non-specific damage or the opening of defined, time-dependent susceptibility windows. We therefore tested whether brief CAP exposure opens antibiotic susceptibility windows in mature *S. aureus* biofilms grown on titanium, an implant-relevant surface, and used time-resolved molecular profiling to define the associated recovery trajectory.

**Methods:** Mature *S. aureus* biofilms formed on titanium were exposed to sub-lethal CAP prior to challenge with clinically relevant antibiotics. Changes in susceptibility were assessed using biofilm-focused viability and metabolic readouts. To define the underlying recovery biology, we integrated time-resolved RNA-seq and proteomics across early damage and recovery phases.

**Results:** CAP did not produce a uniform increase in antibiotic susceptibility. Instead, it generated an antibiotic-specific response pattern in titanium-associated *S. aureus* biofilms. Early after treatment, biofilms entered a low-energy state marked by broad suppression of translation, respiration and ATP-generating functions. Surviving populations then followed a structured recovery trajectory, with marked shutdown at 1 h, partial rebuilding of nucleotide, amino acid and envelope-associated functions by 4 h, and a distinct recovered state by 24 h. Within this recovery window, ciprofloxacin showed the clearest enhancement, whereas  $\beta$ -lactam activity remained comparatively constrained. These drug-specific outcomes were accompanied by differential recovery of pathways linked to DNA repair, efflux, peptidoglycan turnover and autolysis.

**Conclusions:** CAP acts as a physiological primer that opens transient antibiotic susceptibility windows in titanium-associated *S. aureus* biofilms. These findings support a state-based model for CAP-antibiotic scheduling in implant-associated biofilm infection.



## P10.16 - HYBRIDISATION OF CIPROFLOXACIN WITH GERANIOL DISRUPTS STAPHYLOCOCCUS AUREUS BIOFILMS AND OVERCOMES FLUOROQUINOLONE RESISTANCE

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**Background & Aims:** *Staphylococcus aureus* is a major opportunistic pathogen whose ability to form biofilms and develop multidrug resistance significantly compromises the efficacy of antibiotics. Although combination therapies may enhance antibacterial performance, their clinical application is often limited by pharmacokinetic and pharmacodynamic profiles. This study aimed to develop a novel hybrid molecule conjugating ciprofloxacin, a fluoroquinolone antibiotic, with the plant-derived monoterpenoid geraniol (Ger\_CIP) and to evaluate its antibiofilm activity against ciprofloxacin-susceptible and -resistant *S. aureus* strains.

**Methods:** The minimum inhibitory concentration (MIC) of the Ger\_CIP hybrid was determined for *S. aureus* ciprofloxacin (*S. aureus* CECT 976) susceptible and resistant strains (*S. aureus* 8093). Antibiofilm activity was assessed in pre-established biofilms by quantifying biomass (crystal violet), metabolic activity (resazurin), and culturability (colony-forming units, CFU). The impact of Ger\_CIP on biofilm thickness was analysed using optical coherence tomography (OCT).

**Results:** The susceptible strain showed a MIC of 0.125 µg mL<sup>-1</sup> for ciprofloxacin, while the resistant strain exhibited values above 1024 µg mL<sup>-1</sup>. In contrast, Ger\_CIP displayed MIC values of 8 µg mL<sup>-1</sup> in the susceptible strain and 16 µg mL<sup>-1</sup> in the resistant strain. In pre-established biofilms of the resistant strain, where ciprofloxacin was ineffective, Ger\_CIP reduced biomass by up to 40%, inhibited metabolic activity by approximately 90%, and decreased culturability by about 2.3 log CFU cm<sup>-2</sup>. Regarding biofilm structure analysed by OCT, untreated resistant biofilms had a mean thickness of 56 µm and a maximum thickness of 92 µm. Ciprofloxacin reduced the mean and maximum thickness to approximately 26 µm and 48 µm, respectively. Ger\_CIP further reduced the mean thickness to 10 µm and the maximum thickness to 26 µm at 16 µg mL<sup>-1</sup>.

**Conclusions:** Ger\_CIP retains antibacterial activity against a highly ciprofloxacin-resistant *S. aureus* strain and markedly enhances disruption of established biofilms compared with ciprofloxacin alone.



## P10.17 - DECIPHERING THE MECHANISMS OF SYNERGISTIC REPEATED PHOTOACTIVATION OF A NATURALLY DERIVED COMBINATION AGAINST DUAL-SPECIES *PSEUDOMONAS AERUGINOSA* *STAPHYLOCOCCUS AUREUS* BIOFILMS

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**Background & Aims:** Diabetic foot ulcers (DFUs) are often colonised by polymicrobial biofilms dominated by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, whose structural complexity and extracellular matrix reduce antibiotic efficacy. Combining antibiotics with antimicrobial photodynamic inactivation (aPDI) has shown promise in restoring the antibiotic activity. In addition, the use of phytochemicals with intrinsic photosensitising capacity further strengthens this strategy, as these compounds act as antibiotic adjuvants and exert multi-target effects. However, the mode of action underlying this activity against clinically derived polymicrobial biofilms remains insufficiently characterised. This study evaluated the antibiofilm mode of action of blue-light photoactivated berberine-gentamicin (Ber-Gen) combination against *P. aeruginosa* MJMC568-A and *S. aureus* MJMC568-B dual-species biofilms, both isolated from a DFU patient.

**Methods:** Pre-formed dual-species biofilms were treated with a Ber-Gen combination (at 1000 µg/ml-1024 µg/mL) for 2 h and exposed to blue light at 30 mW/cm<sup>2</sup> for 10 min. Irradiation was applied once every 24 h over three consecutive days, mimicking a more clinically realistic treatment. Following the third application, the biofilms were incubated for an additional 72 h to assess their regrowth capacity. Mechanistic analyses of the antibiofilm effect included the structural organisation and spatial distribution, analysed by confocal laser scanning microscopy. Extracellular polymeric substance (EPS) components were evaluated using confocal imaging, together with spectrophotometric and fluorometric assays. Biofilm architecture and thickness were further characterised by optical coherence tomography (OCT).

**Results:** Repeated irradiation cycles using blue light-activated Ber-Gen combination induced cumulative and sustained alterations in dual-species biofilms. Even 72 h after the last irradiation, the OCT analyses showed a sustained reduction in biofilm thickness and surface roughness. Confocal three-dimensional imaging confirmed disruption of biofilm organisation, indicating limited structural recovery. Quantitative analysis of EPS components revealed a significant reduction in extracellular proteins, polysaccharides, and extracellular DNA, indicating disruption of matrix integrity.

**Conclusions:** Repeated blue light activation of the Ber-Gen combination induces sustained structural disruption and cellular damage, limiting regrowth of dual-species biofilms and supporting its potential for DFU treatment.



## P10.18 - NETWORK-LEVEL DIVERGENCE IN CYCLIC DI-GMP SIGNALLING DRIVES ECOLOGICAL VERSATILITY IN ACINETOBACTER BAUMANNII

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**Background & Aims:** *Acinetobacter baumannii* is listed as a critical priority pathogen by the World Health Organization. It is able to form strong biofilms that help it tolerate environmental aggressions, including antimicrobial treatments. However, little is known about how it regulates biofilm formation and virulence. Here, we investigate the role of the second messenger cyclic di-GMP (c-di-GMP) in regulating these virulence behaviours in multidrug resistant (MDR) clinical isolates of *A. baumannii*.

**Methods:** Comparing strains with high versus low c-di-GMP levels, we performed an RNA sequencing experiment. The transcriptomic results were then validated by performing biofilm formation, exopolysaccharide production and twitching motility experiments. C-di-GMP effect on virulence was assessed in vivo. Furthermore, we performed a genomic analysis on the conservation of diguanylate cyclase (DGC) and phosphodiesterase (PDE) enzymes, involved in c-di-GMP regulation, throughout the *A. baumannii* phylogeny using a customised pangenome of >9,600 genomes. Additionally, PdeD role in *A. baumannii* tolerance to hospital- and host-related stresses was tested using bleach and hydrogen peroxide disc diffusion assays, desiccation tolerance and survival in human serum experiments. PdeD effect on *A. baumannii* fitness in vivo was determined by assessing the attachment to human lung epithelial cells, and the survival in macrophages and bacteraemia murine model.

**Results:** Our results confirmed the positive role of elevated c-di-GMP levels in biofilm formation to the detriment of motility and its effect on *A. baumannii* virulence. However, we observed that cells responded to fluctuating c-di-GMP levels in a strain-specific manner. Furthermore, we demonstrate that specific c-di-GMP regulatory enzymes are associated with specific international clones. Importantly, our data revealed PdeD as the primary functional PDE in the clinically significant International Clone I (IC1). Further characterisation of PdeD demonstrated its central regulatory role in the rapid adaptability of MDR *A. baumannii* strains by controlling their biofilm levels and motility, as well as their persistence in hospital settings and host colonisation in the murine model.

**Conclusions:** This work uncovers the regulatory role of c-di-GMP in MDR *A. baumannii* clinical isolates. Our work suggests that PdeD and the ecological versatility it confers may have played a key role in IC1 dissemination, making it a potential novel therapeutic target.



## P10.19 - FOCUS ON ENDOSCOPE REPROCESSING: PERACETIC ACID EFFICACY AGAINST BIOFILMS OF CLINICALLY RELEVANT ESKAPE PATHOGENS

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**Background & Aims:** ESKAPE pathogens play a central role in nosocomial infections and are characterized by high resistance to a wide range of antibiotics. In the past, isolated nosocomial outbreaks have been linked to contaminated endoscopes, even when reprocessing complied with hygiene guidelines. Biofilm formation is a suspected transmission factor, as bacteria embedded in an extracellular matrix are highly tolerant to antimicrobials. Since disinfectant efficacy is mainly tested on planktonic cells, it remains unclear whether this adequately reflects clinically relevant biofilm conditions.

**Methods:** For the standardized cultivation of biofilms of *E. faecium* ATCC 6057, *S. aureus* ATCC 6338, *K. pneumoniae* ATCC 13883, *A. baumannii* ATCC 19606, *P. aeruginosa* ATCC 15442, and *E. cloacae* ATCC 13047, the "Bead Assay for Biofilms" was used. This method enables the cultivation of biofilms on sintered glass beads. The biofilm-colonized beads were disinfected with peracetic acid (PAA) under the manufacturer-specified conditions for PAA-based disinfection in a commercial automated endoscope washer-disinfector (AEWD): Standard (0.075% PAA, 5 min, 37°C) and Intensive (0.15% PAA, 10 min, 37°C). A bacterial count reduction of  $\geq 5 \log_{10}$  was defined as the criteria for successful disinfection.

**Results:** Under standard conditions, no sufficiently high bacterial count reduction ( $1.0\text{--}3.0 \log_{10}$ ) was achieved for biofilms of all ESKAPE pathogens tested. Intensive conditions resulted in a  $\geq 5 \log_{10}$  for the following species: *E. faecium* (5.7), *S. aureus* (6.75), *K. pneumoniae* (5.71), *A. baumannii* (5.42), and *E. cloacae* (5.03). However, no sufficiently high reduction was observed for *P. aeruginosa* (4.25), nor in dual-species biofilms comprising *S. aureus* and *P. aeruginosa* (2.00).

**Conclusions:** PAA disinfection has demonstrated inconsistent efficacy in its capacity to combat biofilm-associated ESKAPE pathogens under standard AEWD conditions. While the reprocessing of endoscopes entails cleaning and rinsing steps that also reduce bacterial load, the present study focused exclusively on the disinfection aspect. Presently, a limited number of European (EN) standards address the standardized cultivation of biofilms for the purpose of disinfectant testing. This limitation hinders the reliable evaluation of product efficacy against biofilms, including in the context of endoscope reprocessing. Consequently, the efficacy of disinfectants in the context of biofilms should be a pivotal consideration in evaluations of their effectiveness.



## P10.20 - ANALYSIS OF MOLECULAR REGULATION AND QUORUM SENSING IN BIOFILMS OF CLINICAL ISOLATES OF KLEBSIELLA PNEUMONIAE AND CONTRIBUTION OF PHOTODYNAMIC INACTIVATION IN COMMUNICATION OF BACTERIA WITHIN BIOFILMS

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**Background & Aims:** Communication between bacteria is essential for biofilm development. Bacteria communicate via *quorum sensing* (QS), which coordinates biofilm aggregation and regulates gene expression. In *Klebsiella pneumoniae*, QS depends on the AI-2 system (*LuxS* gene) mediated intra- and inter-species signaling in biofilm. Eradication of bacterial biofilm has remained serious medical problem. One of the alternative anti- biofilm approaches could be photodynamic inactivation (PDI). The aim of this study was to analyze QS regulation in clinical isolates of *K. pneumoniae* with different virulence and to study regulation of virulence genes (*fimH*, *mrkD*, *entB*) in biofilm. Additionally, this study tested inhibitory effect of PDI against biofilms and determined contribution of PDI in QS and molecular regulation of *K. pneumoniae* biofilm.

**Methods:** Molecular regulation of biofilms was tested by qPCR to determine changes in expression of QS and biofilm- associated genes. Regulation of QS genes was studied in different stages of biofilm. The effectiveness of PDI was tested on 24h biofilms of selected *K. pneumoniae* isolates. Three concentrations 0.5;0.25;0.125 mM of Methylene blue (MB) as a photosensitizer were tested for PDI. The viability of biofilms with/wo PDI were evaluated by counting CFU and qPCR was used to determine changes in the expression of genes related to QS and biofilm of *K. pneumoniae* after PDI.

**Results:** The ability of clinical isolates of *K. pneumoniae* to form biofilm correlated with the regulation of virulence (*fimH*, *mrkD*, *entB*) and QS (*LuxS*, *LsrR*) genes. The highest regulation of all tested genes was observed in strong biofilm producers. The hypermucoviscous *K. pneumoniae* had the highest gene expression of *LuxS* and *mrkD* genes among all isolates indicating its high virulence. Afterwards, two strong biofilm producers (one hypermucoviscous) among *K. pneumoniae* were selected to perform PDI. The PDI was the most effective with concentration of 0.125 mM MB after 1 min of irradiation (red laser). Additionally, qPCR showed a decrease in the relative expression of all genes associated with QS and biofilm in *K. pneumoniae* after PDI.

**Conclusions:** Results confirmed correlation of high QS regulation and biofilm forming capacity of *K. pneumoniae* and pointed to a high anti-biofilm potential of PDI against hypervirulent *K. pneumoniae* as well.



## P10.21 - EVALUATION OF ANTIBIOTIC RESISTANCE IN AUTOAGGREGATED STAPHYLOCOCCUS AUREUS USING A MAGNETIC LEVITATION-BASED EXPERIMENTAL SYSTEM

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**Background & Aims:** Objective. To define the relationship between autoaggregation and antibiotic resistance of gram-positive bacteria *Staphylococcus aureus*. Bacterial aggregation is the formation of clumps embedded in a self-produced extracellular polymeric matrix without surface attachment (Trunk et al., 2018), distinguishing aggregates from classical biofilms (Flemming et al., 2016). While biofilms are established contributors to chronic infections (Chen and Wen, 2011; Zhang et al., 2018), floating aggregates have been implicated in pathogen persistence in bronchitis, otitis, and joint infections (Alhede et al., 2011; S. S. S. Dastgheyb et al., 2015). Biofilm-associated bacteria may display up to 1000-fold increased antibiotic resistance compared to planktonic cells (Anderson and O'Toole, 2008; Hall and Mah, 2017), yet resistance within aggregates remains poorly characterized. Autoaggregation (single-species aggregation) may differ mechanistically from biofilm formation (Chen et al., 2016; Sharma et al., 2017). Investigation is complicated by aggregate sedimentation; therefore, magnetic levitation, compensating gravity by magnetic force, was previously applied to enable long-term autoaggregate cultivation (Parfenov et al., 2018; Domnin et al., 2020, 2022; Ermolaeva et al., 2023).

**Methods:** MSSA strains 129B and 521 and MRSA strain SA180-F were studied. Antibiotic resistance was compared in stationary planktonic cultures, mature surface-attached biofilms, and floating autoaggregates, relative to MIC values obtained in exponential cultures. Autoaggregates were generated using a magnetic levitation system with two neodymium magnets. Bacteria were exposed to 10x MIC of streptomycin or ciprofloxacin. Survival was expressed as the percentage of viable cells compared to antibiotic-free controls. Autoaggregate biomass volume was additionally assessed.

**Results:** Stationary cultures were more resistant than logarithmic cultures. After 24 h at 10x MIC streptomycin, survival was 0.5%, 0.26%, and 0.58% for strains 129B, 521, and MRSA; for ciprofloxacin, 1.14%, 0.24%, and 2.29%, respectively. Streptomycin survival was comparable in MSSA biofilms and autoaggregates, whereas MRSA showed marked differences (72% vs 4.17%). For ciprofloxacin, MSSA survival was higher in biofilms than autoaggregates, while MRSA survival was similar.

**Conclusions:** Biofilms and autoaggregates exhibit strain- and antibiotic-dependent differences in antimicrobial response. Results obtained in biofilm models cannot be directly extrapolated to autoaggregate systems.



## P10.22 - THE GLOBAL TRANSCRIPTOME RESPONSE OF PSEUDOMONAS AERUGINOSA TO EPOXYTIGLIANE EBC-1013

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**Background & Aims:** The epoxytiglane EBC-1013 is a topical wound therapeutic with immunomodulatory and antibiofilm activity that accelerates healing in acute thermal and chronic diabetic wounds. Its antibiofilm effects in *Pseudomonas aeruginosa* are linked to altered virulence and stress-response phenotypes, but the transcriptional mechanisms underlying these shifts remain unclear. In this study, we sought to define the molecular pathways affected by treatment using RNA sequencing (RNAseq).

**Methods:** RNAseq was performed on *P. aeruginosa* PAO1 cultures exposed to EBC-1013 (64, 128, and 256 µg/mL) alongside ethanol vehicle controls (24 h, 37 °C; n = 4). RNA quality and integrity were verified prior to whole-transcriptome Illumina library preparation. Paired-end reads were trimmed and aligned to the *P. aeruginosa* PAO1 reference genome (GCA\_000006765.ASM676v1). Differential gene expression and pathway-level effects were assessed.

**Results:** EBC-1013 at 128 µg/mL induced extensive transcriptional changes ( $P < 0.05$ ), with  $\log_2$  fold-change shifts in oxidative and electrophilic stress responses typified by upregulation of organic hydroperoxide resistance gene *ohr* (+1.34), quinone reductases (*azoR2*, +1.61; *azoR3*, +1.78); denitrification pathways (*nar*, +1.92; *nir*, +1.46; *nor*, +2.27; *nos*, +2.02 families). Down-regulation of specific quorum sensing (QS) genes (*lasI*, -1.27; *pqsH*, -1.82; *rsaL*, -1.94, and *antABC*, involved in the synthesis of Pseudomonas quinolone signal, -3.41) were noted. The global QS negative regulator *qscR* however, was up-regulated (+1.77). Down-regulation of important virulence determinants (*phzB2*, -1.21; *phzC2*, -1.36; *toxA*, -0.96; *toxR*, -1.25; *lecB*, -1.25; *aprA*, -2.27), and iron metabolism/siderophore biosynthesis operons (*pch*, -3.16; and *pvd*, -3.50) were also noted. At 256 µg/mL, changes in the transcriptional profile were less pronounced but showed enhanced activation of anaerobic respiratory pathways (*ccoNOP2*, +1.96), denitrification (*nir* family, +1.94), and stress responses (*ohr*, +1.90; *azoR2*, +0.78). Transcriptomic data were validated by qPCR and HPLC analysis.

**Conclusions:** EBC-1013 demonstrated dose-dependent effects on transcription in *P. aeruginosa* targeting anaerobic adaptation, oxidative and electrophilic stress responses; and coordinated disruption of iron metabolism, quorum sensing, and phenazine biosynthesis. These pathway-level changes provide mechanistic insight into the antibiofilm activity of EBC-1013 and identify virulence-linked regulatory nodes that may underpin observed in-vitro phenotypic responses to treatment. EBC-1013 is currently undergoing evaluation in human phase I studies in chronic venous leg ulcers (ACTRN12624000544572).



## P10.23 - NATURAL MINERAL WATERS AS ADJUNCTIVE STRATEGIES AGAINST MULTIDRUG-RESISTANT PATHOGENS IN BIOFILM-ASSOCIATED DIABETIC FOOT ULCERS

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**Background & Aims:** Diabetic foot ulcers (DFUs) are chronic, biofilm-prone wounds in which multidrug-resistant (MDR) pathogens, inflammatory persistence and extracellular matrix degradation converge to delay healing and increase the risk of severe clinical outcomes. Biofilm-associated infections are particularly difficult to eradicate, as microbial communities tolerate antimicrobial pressure and sustain a hostile wound microenvironment. Natural mineral waters (NMWs), due to their distinctive physicochemical composition, may represent underexplored adjunctive mineral-based control strategies. This study aimed to compare the antimicrobial, anti-inflammatory and extracellular matrix-protective potential of six NMWs from Northern Portugal against key barriers involved in DFU biofilm-associated infections.

**Methods:** Six NMWs (NMW-A to NMW-F) were chemically characterized and evaluated *in vitro* for antimicrobial activity against MDR wound-associated bacteria, including *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Growth inhibition was used as an initial screening endpoint for anti-pathogen activity. Anti-inflammatory potential was assessed using RAW 264.7 macrophages by determining half- maximal effective concentrations (EC<sub>50</sub>). Modulation of the wound enzymatic microenvironment was investigated through collagenase and hyaluronidase activity assays. Associations between mineral composition and bioactivity were explored to identify candidate mineral signatures linked to therapeutic performance.

**Results:** The NMWs displayed selective bioactive profiles against MDR bacteria commonly implicated in DFU biofilm- associated infections. NMW-C showed the highest inhibition of *Staphylococcus aureus* (53%), a key biofilm-forming wound pathogen, while NMW-D was most active against *Klebsiella pneumoniae* (35%). NMW-F was the only water with detectable activity against *Pseudomonas aeruginosa* (9%), a highly tolerant pathogen associated with mature biofilms and antimicrobial recalcitrance. Beyond anti-pathogen effects, NMW-B, NMW-E and NMW-F showed the strongest anti-inflammatory activity, with EC<sub>50</sub> values of 24–25 µg/ml. Selected NMWs also reduced collagenase and hyaluronidase activities, suggesting extracellular matrix protection. These effects were associated with bicarbonate, magnesium, zinc and copper.

**Conclusions:** Specific NMWs from Northern Portugal combine antimicrobial activity against MDR wound pathogens with anti-inflammatory and extracellular matrix-modulating effects. Although further dedicated antibiofilm assays are required, these findings support NMWs as promising adjunctive mineral-based strategies to target microbial and host-derived barriers sustaining DFU biofilm-associated infections, including pathogen tolerance, inflammatory persistence and matrix degradation, in future translational wound-care platforms.



## P10.24 - EXPERIMENTAL EVOLUTION OF ANTIBIOTIC RESISTANCE IN THE EX VIVO PIG LUNG BIOFILM MODEL

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**Background & Aims:** Biofilms occurs in 65% of all infections and 80% of chronic infections. In people with cystic fibrosis (PwCF), where mucus builds up around organs making them more prone to infections, chronic lung infections are driven by biofilm formation. These biofilms are rarely eradicated by antimicrobials in CF increasing the severity of chronic infections. Furthermore, the CF environment is known to cause a high number of hypermutator phenotypes in biofilms that have been shown to frequently become multidrug resistant. Difficulty in treating biofilms in PwCF contributes to a heavy economic burden, costing the world economy \$7.5 billion each year. It is therefore crucial to have a better understanding of the role biofilms play in antimicrobial resistance (AMR) evolution to fully assess AMR acquisition.

**Methods:** To closely mimic the host environment we used the ex vivo porcine lung model (EVPL) to assess the evolution of *P. aeruginosa* biofilms under meropenem selective pressure using an evolutionary ramp approach. We serially passaged *P. aeruginosa* biofilms via colonisation of new tissue pieces for 10 days. We whole genome sequenced evolved clones and assessed their collateral susceptibility to other antibiotics and comparative fitness.

**Results:** We identified that biofilm could easily seed an uninoculated piece of porcine bronchiole from an inoculated piece. Although *P. aeruginosa* biofilms under meropenem selection pressure were observed to initially decrease in size, as determined by light microscopy, they were able to adapt and recover over 10 days. While not clinically resistant, we found that *P. aeruginosa* clones isolated from these biofilms had decreased susceptibility to meropenem and collateral resistance to several other antibiotics. This decreased susceptibility was associated with minimal fitness costs in the absence of antimicrobials. We identified the causal and compensatory mutations using whole genome sequencing.

**Conclusions:** Here, we found it is possible to evolve *P. aeruginosa* biofilms in the EVPL model under meropenem selective pressure and identify the collateral network and associated fitness costs. These differed from previous planktonic selection results. Future antimicrobial testing should take into consideration the altered evolutionary response in biofilms as part of the drug development pipeline to better optimise our current antimicrobials in the clinic.



## P10.25 - PERSISTENT CELL EMERGENCE OCCURS BEYOND MATURE STAGES OF CRYPTOCOCCUS BIOFILMS

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**Background & Aims:** The World Health Organization classifies *Cryptococcus neoformans* (Cn) and *Cryptococcus gattii* (Cg) as priority fungal pathogens. Antifungal resistance significantly worsens the treatment and prognosis of cryptococcosis. Biofilm formation and the presence of persister cells—tolerant subpopulations within biofilms capable of surviving high doses of antifungals, such as amphotericin B (AMB), and adapting their metabolism under stress—represent resistance-associated factors that may be decisive in the failure of aggressive therapeutic approaches, including the use of the fungicidal yet highly toxic AMB, thereby contributing to infection persistence. Objectives: To investigate the presence, temporal dynamics, and antifungal tolerance of persister cells during early and mature stages of biofilm formation by *Cryptococcus neoformans* and *Cryptococcus gattii*.

**Methods:** Biofilms were formed for 2 h and 48 h and subsequently treated with high doses of AMB (100 µg/mL). Colony-forming unit assays, multiphoton microscopy, metabolic activity assessments, and susceptibility tests were performed.

**Results:** During both the pre-adhesion (2 h) and maturation (48 h) phases, persister cells of *C. neoformans* and *C. gattii* were detected in biofilms exhibiting low metabolic activity during treatment with 100 µg/mL AMB as selective pressure. Following antifungal removal, these cells displayed slow growth, indicating the ability to alternate between dormant and metabolically active states, with gradual resumption of proliferation after supplementation with Sabouraud medium. These subpopulations represented a small fraction of the biofilm in microscopy analyses but survived the selective pressure applied during both pre-adhesion and maturation. Susceptibility assays confirmed the tolerant phenotype across species and stages, requiring extremely high concentrations of fluconazole (>500 µg/mL) and amphotericin B (≥ 400 µg/mL).

**Conclusions:** Persister cells emerge in *C. neoformans* and *C. gattii* biofilms from the pre-adhesion stage and persist through maturation, exhibiting metabolic switching between induction and recovery and high antifungal tolerance, indicating that biofilm recalcitrance is an intrinsic phenomenon rather than being restricted to late developmental stages.



## P10.26 - PHYTOCHEMICALS AS ALTERNATIVE STRATEGIES TO TRADITIONAL BIOCIDES FOR CONTROLLING BACTERIAL BIOFILMS IN HOSPITAL DISINFECTION

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**Background & Aims:** The use of biocides has increased rapidly in a wide variety of applications. This is a major public health issue, since the intense usage could lead to bacterial resistance and cross-resistance with unrelated antimicrobials, including antibiotics. Biofilms, complex adherent and structured microbial communities, aggravate this problem because they provide a protective environment for bacteria. Phytochemicals are effective, safe, inexpensive, and broad-spectrum antibacterial agents.

**Methods:** This study evaluated the antibiofilm activity of two traditional biocides, Benzalkonium Chloride (BAC) and Peracetic Acid (PAA), and two phytochemicals with promising antimicrobial properties, Salicylic Acid (SAL) and Eugenol (EUG). First, the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined for all molecules against *Escherichia coli* CECT 434. Additionally, reduction in biomass (crystal violet staining), metabolic activity (alamar blue staining), and in cell culturability were assessed in 24h biofilms after 30 min of exposure to each molecule (at MIC and 10×MIC). The same methodology was also applied for dual and triple combinations with one biocide and one or two phytochemicals against *E. coli* biofilms.

**Results:** BAC showed the lowest MIC and MBC (12.8 and 25.6 mg/L), followed by PAA (240 and 480 mg/L), EUG (800 and 1600 mg/mL), and SAL with the highest values (3200 and 6400 mg/mL). When tested against 24 h *E. coli* biofilms, most treatments resulted in low to moderate biomass reduction. BAC and PAA at 1×MIC reduced biomass by 15% to 18%, while 10×MIC achieved reductions of almost 20%. SAL at 10×MIC showed the highest biomass removal ability, reaching approximately 35% reduction, whereas EUG at 10×MIC achieved around 10% reduction. Regarding metabolic activity effects, BAC and PAA revealed, in general, low efficacy. Notably, phytochemicals demonstrated competitive performance, with SAL and EUG, at 10×MIC, achieving reductions of approximately 90% and 30%, respectively. Culturability assays demonstrated significant reductions of culturable biofilm cells, ranging from 1.5 log CFU/cm for BAC to 4.5 log CFU/cm<sup>2</sup>. Interestingly, the combinations significantly improved reductions in biomass, metabolic activity, and culturability.

**Conclusions:** These results suggest that current disinfection protocols may be insufficient to overcome biofilm recalcitrance and highlight the potential of natural molecules, phytochemicals, as alternatives or adjuvants to biocides.



## P10.27 - CEFIDEROCOL EFFICACY IN PSEUDOMONAS AERUGINOSA BIOFILMS: IMPACT OF IRON LIMITATION, SIDEROPHORE TRANSPORT AND METALLO- $\beta$ -LACTAMASE PRODUCTION

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**Background & Aims:** *Pseudomonas aeruginosa* (PA) relies on siderophore-mediated iron uptake to support biofilm formation. Siderophore-cephalosporins such as cefiderocol (FDC), one of the most relevant options against metallo- $\beta$ -lactamase (MBL)-producing strains, exploit this pathway. However resistance to FDC has been linked to alterations in iron-uptake systems, particularly PiuA/DC. Given the current knowledge gap, here we evaluated FDC activity in biofilms, and analysed whether it is affected by iron limitation, mutations of siderophore transport systems (PiuC) and MBL production (VIM-2).

**Methods:** Reference strain PAO1DK and its isogenic mutants PAO1DK $\Delta$ piuC, PAODK+pUCP-VIM-2 and the double mutant PAODK $\Delta$ piuC+pUCP-VIM-2 were used. MIC and MBCs of FDC were determined by microdilution. Biofilm bactericidal concentration (BBC), defined as the lowest concentration of the antibiotic producing a  $\geq 3$  log reduction in bacterial load compared with untreated control biofilms, was assessed using a modified Calgary Biofilm Device. All susceptibility testing were performed in cation-adjusted Mueller-Hinton (CA-MH) and iron-depleted (ID-CAMHB) to evaluate the impact of iron availability.

**Results:** FDC MICs values in CAMH were generally 2 or 4-fold higher comparing with ID-CAMH. MBCs were 2 or 3-fold higher in CAMH. The double mutant PAODK $\Delta$ piuC+pUCP-VIM-2 reached an MIC of 8  $\mu$ g/mL, resistant according to EUCAST breakpoints. Biofilm bactericidal activity was enhanced under iron depletion, with BBCs of 8  $\mu$ g/mL versus  $\geq 64$   $\mu$ g/mL in CAMH. In iron-depleted conditions, FDC concentrations above the BBC determined a lower killing for derivatives producing VIM-2 +/- the *piuC* mutation than for the wildtype. No differences between strains were observed in CAMH.

**Conclusions:** FDC fails to eradicate *P. aeruginosa* biofilms in static models regardless of iron availability. However, as previously noted for planktonic growth, FDC activity is shown to be enhanced under iron-depleted conditions in biofilms. Moreover, production of MBLs and mutations in iron transport systems reduce to some extent FDC activity against biofilms, but at concentrations above BBC.



## **P10.28 - SPATIOTEMPORAL DEVELOPMENT OF ANTIBIOTIC PERSISTENCE IN VIBRIO CHOLERAE BIOFILMS DEPENDS ON EMERGENT MICROENVIRONMENTS**

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**Background & Aims:** Bacteria living in biofilm populations have a significantly higher chance of survival after antibiotic treatment, compared to bacteria in planktonic populations. Biofilms are characterized by complex spatiotemporal gradients, while planktonic cultures represent a relatively homogenous system. However, the mechanistic link between this structural heterogeneity and the resulting antibiotic treatment outcome remains poorly defined. Our study shows that *V. cholerae* biofilms survive antibiotic treatment by a deterministic process, explained by emergent biofilm microenvironments.

**Methods:** Using confocal microscopy, we quantified spatial gradients of survival frequency within 3D biofilms across different growth stages, and used spatial transcriptomics and perturbation experiments to identify causative microenvironments.

**Results:** We observed that the probability of antibiotic persistence, a key phenomenon in treatment survival, appeared exclusively in biofilms above a certain size, and in spatial niches distant from the biofilm periphery. Among the different microenvironments prevailing in these niches, neutralising low pH and anoxia led to a significant reduction in survival. Furthermore, we established that biofilm survival is not absolute, and can be systematically eradicated using consecutive, temporally spaced periods of antibiotic treatment.

**Conclusions:** Our findings successfully decouple the contributions of antibiotic penetration, biofilm development, and influence of microenvironments on survival outcome in biofilms. This study provides a mechanistic understanding for the inherent resilience of biofilms and offers a rigorous framework for understanding the implications of phenotypic heterogeneity in multicellular bacterial systems.



## P10.29 - DISRUPTING BIOFILM-ASSOCIATED GENES REDUCES MYCOBACTERIAL VIRULENCE

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**Background & Aims:** Tuberculosis (TB) is a relentless global threat that, despite extensive global efforts, lacks effective therapeutic options. TB treatment efficacy could be improved by targeting the drug tolerant bacterial subpopulations residing in biofilms. However, mycobacterial *in vivo* biofilms remain poorly characterized. Therefore, we aim to elucidate the molecular mechanisms that regulate biofilm formation in mycobacteria by conducting a genetic screen in *Mycobacterium marinum*, with the objective of identifying potential targets for drug development.

**Methods:** We use *M. marinum*, which is closely related to *Mycobacterium tuberculosis*, as a model pathogen since it causes TB-like disease in fish and can thus be utilized in our zebrafish TB model. We screened 4,200 *M. marinum*  $\phi$ MycoMar T7 transposon mutants on Congo red agar to find those with altering coloration or colony morphology. The first screen identified 158 morphology mutants with potential defects in biofilm formation. These mutants were sequenced to identify the disrupted genes. To discover biofilm-defective mutants among the morphology mutants, eDNA, proteins, and cellulose-like polysaccharides were quantified from the extracellular matrix (ECM) of the morphology mutants. Biomass per cell was determined using crystal violet staining. Lastly, *in vivo* virulence of the selected mutants was evaluated in the zebrafish TB model.

**Results:** Bioinformatic analyses of the 158 transposon mutants revealed two significantly enriched gene clusters among the biofilm mutants harboring genes associated with fatty acid biosynthesis and protein secretion. 20 mutants with the most prominent alterations in the amount of biomass or individual ECM components compared to wild type were tested in the zebrafish TB-model for virulence. The majority of the selected mutants had reduced virulence compared to wild type. A very interesting virulence-defective mutant with transposon insertion in a gene encoding a conserved protein of unknown function was also found. The newly identified virulence factor will be investigated further as a potential drug target.

**Conclusions:** Our results indicate that disruptions in genes associated with mycobacterial biofilm formation reduce virulence. The results strengthen the hypothesis that biofilm formation during mycobacterial infections is essential for infection progression. Thus, targeting biofilm components could be an innovative treatment option for TB.



## P10.30 - CHARACTERIZATION OF A NOVEL PUTATIVE AMYLOID IDENTIFIED IN MYCOBACTERIAL BIOFILM

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**Background & Aims:** Amyloidogenic proteins are an important structural component of many bacterial biofilms, providing robustness and contributing to antimicrobial tolerance. There is currently a considerable lack of knowledge on mycobacterial amyloids, and it is of significance to study these special proteins to better understand the nature of mycobacterial biofilms. In this study we set out to characterize and show the amyloidogenic properties of a SIR2-like domain-containing protein identified by our group in *Mycobacterium marinum* biofilm.

**Methods:** Our group discovered the SIR2-like domain-containing protein in 3-week-old *M. marinum* biofilm after harsh treatments designed to degrade non-amyloidogenic proteins. The protein resisted treatments with SDS and heat, and it was visible on gel after monomerization by trifluoroacetic acid. A single peptide sequence was identified from the gel band with mass spectrometry analysis. The matching protein was predicted to have amyloidogenic regions according to AmyloGram, PASTA 2.0, and Waltz. We have designed recombinant protein constructs based on the sequence analysis, one containing the full-length protein, and three truncated versions for mapping amyloidogenic regions. We will test the aggregation propensity of these constructs using conditions reported for other known amyloids and analyzing the particle size change with dynamic light scattering. The constructs will be further subjected to Congo Red staining, Thioflavin T fluorescence, SDD-AGE, and X-ray fiber diffraction to confirm the presence of amyloid structures.

**Results:** We have identified a heat- and SDS-resistant, trifluoroacetic acid-soluble protein from *M. marinum* biofilm. The amino acid sequence data predicts the protein's amyloidogenicity. We have successfully produced and purified all recombinant proteins we designed according to analysis of the protein sequence. Based on computational analyses, we are confident that our protein of interest will form amyloid fibrils under the right conditions, and we will see the evidence for this as the characteristic Thioflavin T fluorescence and birefringence.

**Conclusions:** The outcome of this study will provide pioneering insight into the nature of mycobacterial biofilms through the discovery of mycobacterial amyloids. The knowledge gained from this research can also be applied to other bacteria to advance studies on biofilm-related pathogenicity.



## P10.31 - DISRUPTION OF CANDIDA SPP. BIOFILMS BY INSECT-DERIVED PEPTIDES: AN IN VITRO STUDY

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**Background & Aims:** The global rise in antifungal resistance and the increasing incidence of *Candida* spp. infections pose a significant clinical challenge, emphasizing the urgent need for alternative therapeutic strategies. Natural compounds, particularly insect-derived peptides, have emerged as promising bioactive agents with antimicrobial potential. This study aimed to evaluate the antifungal activity of insect-derived peptides - Blap-6, isolated from *Blaps rhynchopetera* and Gomesin, isolated from *Acanthoscurria gomesiana* -, against *Candida* spp., using a combined *in silico* and *in vitro* approach.

**Methods:** A total of 37 insect-derived peptides were screened through molecular docking to assess their interactions with key enzymatic targets of *Candida albicans*, including lanosterol 14-demethylase (LDM), secreted aspartic proteinase-5 (Sap-5), N-myristoyltransferase (NMT), and dihydrofolate reductase (DHFR). *In vitro* antifungal activity was evaluated by determining the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) in both planktonic cells and biofilms of ATCC strains of *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*. Biofilm structural changes after treatment were further analyzed using Confocal Laser Scanning Microscopy (CLSM). Biomass production quantification was performed by Crystal Violet staining assay.

**Results:** Docking analysis identified Blap-6 and Gomesin as the most promising candidates. In *in vitro* assays, Gomesin achieved complete biofilm eradication in three of the four *Candida* species tested, while Blap-6 showed moderate but consistent reductions across all species. Notably, *C. tropicalis* demonstrated resistance to complete eradication by both peptides. Three-dimensional biofilm analysis revealed that *C. albicans* exhibited the greatest loss of structural integrity, with absence of intact cells. In contrast, *C. glabrata* showed resistance at the extracellular matrix level, with limited disruption and preserved cellular structure.

**Conclusions:** These findings highlight insect-derived peptides, particularly Gomesin and Blap-6, as promising antifungal candidates. Their activity against *Candida* spp., especially in biofilm conditions, supports their potential as alternative therapeutic agents or as templates for the development of novel peptide-based antifungal drugs.



### **P11.1 - URINARY SECRETORY IGA AND IGG RESPONSES AND INFLAMMATORY MARKERS IN PATIENTS WITH BIOFILM FORMING PSEUDOMONAS AERUGINOSA URINARY TRACT INFECTIONS**

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**Background & Aims:** *Pseudomonas aeruginosa* is a highly biofilm forming uropathogen frequently implicated in catheter associated and recurrent urinary tract infections (UTIs). Biofilm formation promotes prolonged mucosal antigen exposure, immune evasion, and antibiotic tolerance, yet non invasive biomarkers that reflect biofilm associated infection dynamics are lacking. Identifying urinary biomarkers may improve the understanding and diagnosis of *P. aeruginosa* UTIs.

**Methods:** Urine samples were obtained from patients with *P. aeruginosa* positive cultures (n=28), healthy controls (n=23), culture negative hospitalized patients (n=25), and patients with *Escherichia coli* or *Enterococcus faecalis* UTIs (n=27). Anti *P. aeruginosa* secretory IgA (s IgA) and IgG, along with cytokines and proteins associated with mucosal and neutrophil driven responses (Chitinase 3 like protein 1, IL 1 $\beta$ , IL 17, IL 6, IL 8, EGF, VEGF, uromodulin, lipocalin, and IL 5), were quantified using ELISA and Luminex assays.

**Results:** Among *P. aeruginosa* positive samples, 57.1% were from catheterized patients and 35.7% from patients with recurrent UTI (rUTI). Anti *P. aeruginosa* s IgA and IgG levels were significantly elevated in *P. aeruginosa* cases compared with healthy controls (s IgA p<0.001; IgG p<0.0001). No significant differences were observed between single episode UTIs and rUTIs within the *P. aeruginosa* positive group. However, s IgA was elevated only in patients with multiple *P. aeruginosa* positive rUTI samples compared with both healthy (p=0.0034) and culture negative controls (p=0.0228), a pattern not seen in single episode cases. IL 8, lipocalin, and IL 6 were also significantly increased in *P. aeruginosa* positive urine samples relative to healthy controls.

**Conclusions:** Urinary anti *P. aeruginosa* s IgA and IgG are elevated in patients with *P. aeruginosa* UTIs, with s IgA best reflecting infection status and consistent with prolonged mucosal antigen exposure characteristic of biofilm persistence. IL 8, lipocalin, and IL 6 may serve as additional indicators of UTI. More stringent patient selection may further clarify immune response patterns in bio-film related UTIs.



## P11.2 - BONE MICROENVIRONMENT PROMOTES CUTIBACTERIUM ACNES ADHESION AND BIOFILM FORMATION ON TITANIUM

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**Background & Aims:** Prosthesis joint infections (PJI) remain a major clinical challenge, and understanding their pathophysiology is crucial to improve prevention and treatment. Among PJIs, 10% are caused by *Cutibacterium acnes*, a Gram-positive skin colonizer that involves weak inflammation response, complicating early diagnosis of these PJIs. Although *C. acnes* adhesion to prosthetic materials such as titanium has been described, interactions with bone and osteoblasts remain poorly understood. This study investigated the adhesion of *C. acnes* to bone explant and evaluated the impact of bone, osteoblasts, and their secretions on *C. acnes* adhesion and biofilm formation on titanium.

**Methods:** Two clinical strains, C2 (non-PJI isolate) and PJI2 (PJI-isolate), were incubated with fresh (called "cellularized") or frozen (called "cell-free") bone explants, fibronectin-coated titanium disks, primary osteoblasts (OBs) or their secretions, and combinations thereof. Results were obtained through CFU enumeration, microscopies and RT-qPCR.

**Results:** Both strains adhered to bone. For strain C2, adhesion to cellularized bone was associated with increased expression of stress- and biofilm-related genes (*groEL*, *tuf*, *fbaA*, *eno*, *sucD*), and a 3.3-fold increase in adherent bacterial counts compared with cell-free bone, suggesting that bone cells enhanced adhesion. Moreover, the artificial addition of OBs on cell-free bone induced higher bacterial adhesion percentages (39.5%) than in their absence (9.7%). Adhesion to titanium (mimicking prosthesis) was increased in the presence of cellularized bone (3.3- and 4.2-fold for C2 and PJI2, respectively). Bone cells presence did not modify biofilm matrix composition on titanium. However, increased expression of *recA* and *fumC* suggested an adaptive response of *C. acnes* to oxidative stress. Furthermore, OBs supernatant associated with cell-free bone also increased bacterial adhesion percentage to titanium (10.8 % for C2 and 15.4% for PJI2 versus 5.3% and 6.7% without supernatant), indicating that bone cell secretion promoted bacterial adhesion. Cell-free bone also modulated *C. acnes* behavior on titanium, decreasing adherent bacteria counts for C2 (3.4-fold) but increasing them for PJI2 (2.8-fold), highlighting that other bone components may also influence biofilm formation.

**Conclusions:** In conclusion, these findings suggest that both bone mineral matrix and osteoblasts promote *C. acnes* adhesion and biofilm formation, with strain- and OB donor-specific effects suggesting individual-dependent susceptibility to *C. acnes* colonization.



### **P11.3 - STAPHYLOCOCCUS AUREUS FORMS SURFACE-ASSOCIATED Z-DNA THAT PROMOTES A POTENT PROINFLAMMATORY RESPONSE IN THP-1 MACROPHAGES.**

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**Background & Aims:** Extracellular DNA (eDNA) is a key component of the extracellular matrix of many biofilms. Recent studies show that eDNA in biofilms forms non-canonical structures, including Z-DNA and G-quadruplexes (G4). Z-DNA is resistant to degradation by human DNase I and may therefore accumulate in the body. Z-DNA is also immunostimulatory and can induce the production of anti-Z-DNA antibodies. Such antibody production is seen in patients with systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) – autoimmune diseases associated with bacterial infections. We hypothesize that the presence of anti-Z-DNA antibodies may indicate exposure to extracellular Z-DNA, potentially originating from bacterial biofilms. The aim was to describe the pathways for immune activation by Z-DNA and G4 in bacterial biofilms, and to determine if this cell-free Z-DNA can cause flare-up of autoimmune diseases.

**Methods:** *Staphylococcus aureus* (*S. aureus*) was grown for 72h to induce Z-DNA production, while exponential phase *S. aureus* devoid of Z-DNA were used as controls. Surface-associated Z-DNA and G4-DNA were visualized by confocal laser scanning microscopy using the antibodies Z22 and BG4. Control samples with synthetic Z-DNA was formed with brominated dsDNA or non-brominated dsDNA complexed with chitosan. G4 was formed with a guanine-rich ssDNA oligo. THP-1 macrophages were cultured and then stimulated with *S. aureus*-associated and free-form Z-DNA and/or G4. CXCL10, a chemokine induced by both STING-dependent and -independent pathways during infection and inflammation, promoting recruitment of effector cells, was quantified by ELISA.

**Results:** Microscopy revealed the presence of cell-surface-associated Z-DNA and G4 in 72h *S. aureus* aggregates, whereas neither structure was detected in exponentially growing *S. aureus*. Preliminary results show that free-form Z-DNA induced a proinflammatory response in THP-1 macrophages measured by CXCL10. Stimulation with 72h *S. aureus* cultures resulted in significantly higher CXCL10 production by THP-1 macrophages compared with exponentially growing *S. aureus*, indicating that bacterial Z-DNA and G4-DNA induces a proinflammatory immune response.

**Conclusions:** THP-1 macrophage proinflammatory responses are highly dependent on the conformation of eDNA. *S. aureus* produces surface-associated Z-DNA and G4 in the late stationary phase. *S. aureus* with surface-associated Z-DNA and G4 enhanced CXCL10 production compared with exponentially growing *S. aureus* lacking surface-associated DNA.



## P11.4 - EX VIVO NAIL MODEL ON THE UNRAVELING OF HOST-PATHOGEN INTERACTIONS OF MONO AND POLYMICROBIAL BIOFILMS OF TRICHOPHYTON RUBRUM ON ONYCHOMYCOSIS AND INVOLVED GENES

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**Background & Aims:** Dermatophyte fungi, like *Trichophyton rubrum*, are major causative agents of skin and nail infections, targeting keratinized tissues. These infections are often associated with biofilm formation, which enhances antifungal resistance through a protective extracellular matrix and may involve interactions with bacteria like *Staphylococcus epidermidis*, leading to persistent polymicrobial infections. However, the mechanisms underlying adhesion, invasion, and biofilm-related pathogenesis in mono and polymicrobial dermatophyte infections remain poorly understood. This study, therefore, seeks to investigate the pathogenesis of these biofilms using *ex vivo* models.

**Methods:** *T. rubrum* ATCC MYA 4438 was cultivated in malt extract agar for 7 days and *S. epidermidis* ATCC 14990 was cultivated in tryptic soy agar for 24 hours. Initially, a fungal inoculum at  $1 \times 10^6$  cells/mL in saline solution was added to the wells of a 96-well microplate, incubated at 37 °C for 3 hours. The supernatant was removed and replaced with a bacterial inoculum of  $1 \times 10^8$  cels/mL in RPMI medium and incubated at 37°C for 72 hours. The resulting biofilm's biomass and metabolic activity were gauged through crystal violet and tetrazolium salt reduction (XTT®), respectively. Sterilized Human Nails were infected, and the colonization of the microorganisms on the model was assessed through Scanning Electron Microscopy and Confocal Microscopy. Relative expressions of *T. rubrum* virulence genes were measured in various conditions through qPCR.

**Results:** The polymicrobial biofilm exhibited behavior distinct from that of the monomicrobial *T. rubrum* biofilm, highlighting interactions among the microorganisms. The sterilized Human Nails model was completely colonized by mono- and polymicrobial infections, with an abundant hyphal network and extracellular matrix surrounding it in both conditions, highlighting the formation of a fungal biofilm on top of bacterial growth. Relative MEP-5 gene expression was increased during monomicrobial nail infection and substantially reduced during polymicrobial infection.

**Conclusions:** Both the characterization of polymicrobial biofilms and qPCR revealed an antagonistic effect on polymicrobial infection by *T. rubrum*. The nails have been shown to be a suitable model for onychomycosis studies, with ample biofilm formation. The MEP-5 gene appears to be a potential biomarker of infection and a target for developing a new therapy for persistent dermatophytosis.



## P11.5 - BIOFILM FORMATION AS A LINEAGE-ASSOCIATED TRAIT IN INVASIVE EXTRAINTESTINAL PATHOGENIC *ESCHERICHIA COLI*

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**Background & Aims:** Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a leading cause of bloodstream infections (BSIs), with ~50% arising from the urinary tract. Although ExPEC is genetically diverse, invasive disease is disproportionately caused by a limited number of successful clones (sequence types, STs). Biofilm formation is considered a key virulence-associated trait, yet population-level comparisons of adherent biofilm production across lineages remain scarce. We tested the hypothesis that biofilm formation is equally distributed across lineages and then evaluated whether enhanced biofilm production associates with the epidemiological success of invasive ExPEC.

**Methods:** Using a curated database of known biofilm-related genes, we characterized 3,254 BSI-derived *E. coli* genomes, generating their biofilm-associated genetic profiles. A subset of 1,083 isolates spanning all eight phylogroups and 218 STs (19 STs with n>10) were selected to represent the genetic diversity in the collection and in vitro quantification of adherent biofilm formation using the Christensen microtiter assay was performed. Curli and cellulose expression were assessed in a subset at 37°C using Congo red and calcofluor white agar.

**Results:** Biofilm-associated genes were enriched in the major ExPEC phylogroup B2 and strongly associated with the most prevalent BSI-associated STs. Across the collection, biofilm biomass showed a left-skewed distribution, indicating that high-level producers constitute a minority. However, lineage-stratified analyses revealed pronounced heterogeneity. The highly prevalent ST73 and ST95 produced the greatest biofilm biomass. In contrast, ST69 exhibited low biomass comparable to commensal-associated ST10 and the pandemic ST131 lineage overall. Notably, within ST131, the multidrug-resistant C1 and C2 subclades produced significantly more biofilm than subclades A and B (Kruskal-Wallis with Dunn's multiple comparisons test,  $p < 0.05$ ). Biofilm biomass was not associated with curli or cellulose expression.

**Conclusions:** Adherent biofilm formation is unevenly distributed across invasive ExPEC lineages. The presented data suggest that the successful ExPEC genetic lineages ST73 and ST95, frequently isolated from BSIs, produce more adherent biofilm when compared to less prevalent and commensal counterparts. These findings raise the question of whether enhanced biofilm formation contributes to lineage success by promoting colonization and/or invasive infection.



## P11.6 - STAGE-RESOLVED ANALYSIS OF MUCOSA-ASSOCIATED BIOFILMS ON ELECTROSPUN HOST-MIMETIC SCAFFOLDS

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**Background & Aims:** The mucosal lining constitutes a critical host interface governing structural organization, nutrient exchange, and surface dynamics at the boundary between tissue and external environment. Biofilms at these mucosal surfaces pose a major clinical challenge due to their persistence and elevated antimicrobial resistance. However, most *in vitro* biofilm models rely on inert substrates and fail to recapitulate the complexity of these mucosal niches, thus limiting their physiological relevance. As a result, mucosa-associated biofilms remain insufficiently characterized, particularly with respect to their structural organization and maturation dynamics under host-relevant conditions. Therefore, the development of robust host-mimetic *in vitro* models is essential to enable mechanistic insight and to allow clinically relevant antimicrobial testing.

**Methods:** To mimic the mucosal host-pathogen niche, electrospun scaffolds were functionalized with porcine mucin, a well-established surrogate of human mucus due to its comparable biochemical properties. Mucin incorporation was verified by ATR-IR spectroscopy and visualized via Alcian blue staining. The scaffolds were subsequently incubated with the clinically relevant pathogen *Escherichia coli* to initiate biofilm formation. Biofilm development and maturation were examined at distinct developmental stages using scanning electron microscopy and confocal laser scanning microscopy. Biofilm mechanics were assessed by nanoindentation, while flow cytometry was employed to evaluate metabolic differentiation, biofilm physiology, and extracellular polymeric substance (EPS) production.

**Results:** Electrospun scaffolds were successfully functionalized with mucin and supported robust biofilm formation with distinct differentiation of early, intermediate, and mature developmental stages. Early stages were characterized by bacterial adhesion and microcolony formation, while intermediate stages exhibited increased biomass accumulation, progressive differentiation, and the emergence of spatial heterogeneity. Mature biofilms displayed an increased EPS production with shifts in the live/dead ratio, indicative of stratification within the bacterial community. Nanoindentation measurements revealed a time-dependent increase in biofilm stiffness, reflecting structural remodeling of the bacterial matrix. Flow cytometry analysis suggested a stage-dependent metabolic differentiation, with including dynamic changes in EPS production.

**Conclusions:** Collectively, we present a robust and physiologically relevant *in vitro* model for the investigation of mucosa-associated biofilms based on mucin-functionalized electrospun scaffolds. Multi-modal imaging and analysis enabled stage-resolved, multidimensional characterization of biofilm maturation and functional evaluation of biofilm-associated processes, including antimicrobial testing under host-mimetic conditions.



## P11.8 - IMPACT OF *S. AUREUS* VIRULENCE ON PATHOGENESIS AND HOST IMMUNE RESPONSE IN *G. MELLONELLA* BIOFILM-MEDIATED IMPLANT- ASSOCIATED INFECTION MODELS

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**Background & Aims:** *Staphylococcus aureus*, the most commonly identified bacterial species in fracture-related infections (FRI), is associated with heterogeneous clinical outcomes, ranging from acute infections, with systemic dissemination to chronic infections characterized by reduced inflammatory symptoms but long-term persistence. While the *S. aureus* virulence profile plays a key role in infection severity, the underlying transcriptional dynamics during implant-related infections remain poorly documented. This study aims to investigate virulence-associated gene expression dynamics using two *S. aureus* isolates, EDCC 5458 and EDCC 5464, responsible for distinct clinical phenotypes, acute and chronic FRI, respectively.

**Methods:** To assess virulence modulation and immunogenicity of both *S. aureus* strains, RT-qPCR was performed to quantify the expression of virulence-associated genes (*hlgA*, *hlgB*, *hlgC*, *scn*, *sak*, *aur*, and *sarA*) and host immune-related markers (*cecA*, *glov1-4*, *hdd11*, *hem*, *lys1-2*, and *PGAB*). Pathogenesis was characterized by monitoring *Galleria mellonella* larval survival over 5 days and by quantifying bacterial dissemination within host tissues and on the implant surface at 24 h post-infection. *G. mellonella* infection models were used to mimic systemic, haematogenous, and biofilm-related infections. Host immune responses were assessed at 24 h post-infection. Biofilm formation on implants was analysed by scanning electron microscopy before and after implantation.

**Results:** No significant differences in larval survival were observed between strains in haematogenous or systemic infection models ( $p > 0.05$ ), whereas biofilm-related infection resulted in significantly lower larval survival in the 5458-infected group (13.3 %) compared with the 5464-infected group (46.6%;  $p \leq 0.01$ ). Both strains exhibited reduced virulence gene expression during biofilm formation; however, a pronounced strain-dependent difference emerged after in vivo maturation, with  $\geq 2.5$ -fold higher expression levels of the  $\gamma$ -haemolysin genes (*hlgA*, *hlgB*, and *hlgC*) in strain 5458 compared with 5464. Moreover, strain 5458 showed a trend toward systemic proliferation, with higher CFU loads per implant (0.5-2.0 log<sub>10</sub> CFU/implant) and in surrounding tissues (0.3-1.0 log<sub>10</sub> CFU/larva;  $p < 0.05$ ), which correlated with a stronger host immune response.

**Conclusions:** These findings reveal infection-dependent transcriptional remodelling consistent with persistence, immune modulation, and bacterial survival strategies. Characterizing *S. aureus* gene expression dynamics during FRI provides insights into host-pathogen interactions and supports the future development of anti-virulence therapeutic approaches.



### **P11.9 - CELLS DERIVED FROM BIOFILM FORMED IN THE PULMONARY MICROENVIRONMENT EXHIBIT DIFFERENTIATED VIRULENCE IN THE GALLERIA MELLONELLA MODEL.**

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**Background & Aims:** *Cryptococcus neoformans* has been categorized by the World Health Organization as a priority fungal pathogen. One of its characteristics is the ability to form biofilms that are often resistant to the drugs used, in addition to representing a potential contributor to its virulence. Thus, the objective of this research was to verify the ability to form biofilms in a pulmonary microenvironment 3D (spheroids) and to verify whether these cells present a differentiated virulent profile when compared to biofilms in atmospheric conditions.

**Methods:** The strains of *C. neoformans* ATCC 208821 and *C. neoformans* clinical isolates from human bronchoalveolar lavage (HBAL) were used to form biofilms under atmospheric + 37°C and pulmonary spheroids conditions (5% CO<sub>2</sub>) + 37°C for up to 120 hours. Subsequently, cells from these biofilms were used to infect a *Galleria mellonella*, and their survival was analyzed over a period of 120 hours.

**Results:** The biofilm of the *C. neoformans* HBAL and the ATCC 208821 reference strain showed high metabolic activity in both pulmonary and atmospheric conditions. The fungal biomass formed also showed similar results for both strains analyzed. However, for the extracellular matrix, the ATCC 208821 strain showed an increase up to 72 hours with a sharp decline at the end of the 120-hour analysis period, while the clinical isolate showed an increase up to 48 hours, with a slight decline at 72 hours and, interestingly, there was an increase in the matrix from 96 hours to 120 hours. Finally, it was observed that *G. mellonella* larvae, when infected by cells from biofilms formed in a pulmonary atmosphere, showed a survival rate of around 10 – 20% when compared to infection caused by cells from biofilms formed under atmospheric conditions, which resulted in a survival rate of 30 – 60%.

**Conclusions:** The tested strains were able to form biofilms under conditions that mimic the pulmonary microenvironment, and spheroids demonstrated adaptations for significant virulence in an in vivo model of *G. mellonella*. These findings demonstrate the need to develop human models that more closely resemble pulmonary physiology to study the adaptive capacity of *C. neoformans* strains derived from biofilms and their virulence.



## P11.10 - THE IMPORTANCE OF UMOB GENE IN BIOFILM FORMATION AND HOST-PATHOGEN INTERACTIONS IN PROTEUS MIRABILIS

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**Background & Aims:** *Proteus mirabilis* is a major uropathogen associated with catheter-associated urinary tract infections (CAUTIs). A key virulence trait of this species is its ability to form crystalline biofilms on urinary catheters, leading to obstruction and persistent infection. The *umoB* gene is known to be involved in the regulation of swarming motility; however, its broader role in *P. mirabilis* physiology and pathogenicity remains poorly understood. This study aimed to investigate the impact of *umoB* gene deletion on *P. mirabilis* pathogenicity, with particular focus on biofilm formation and interaction with host epithelial cells.

**Methods:** An *umoB* knockout mutant was generated in the *P. mirabilis* HI4320 strain using the TargeTron system. The mutant was assessed for growth kinetics in LB medium and swarming motility on agar plates. Biofilm formation was quantified using crystal violet staining and further characterized by fluorescence microscopy. Host-pathogen interactions were evaluated using T24 human bladder epithelial cells, including adhesion and invasion assays.

**Results:** Deletion of *umoB* did not affect bacterial growth compared to the wild-type strain. However, the mutant exhibited altered motility (consistent with the known role of *umoB*) and a significant increase in biofilm biomass as determined by crystal violet staining. This was further supported by fluorescence microscopy, which demonstrated pronounced alterations in biofilm architecture. While the wild-type strain formed a relatively uniform and dispersed biofilm, the *umoB* mutant exhibited dense, heterogeneous aggregates with clearly defined clusters, indicating enhanced structural organization and increased biomass. In host interaction assays, the *umoB* mutant demonstrated significantly increased adhesion to T24 cells compared to the wild type. In contrast, its ability to invade host cells was markedly reduced.

**Conclusions:** Our findings indicate that *umoB* plays a role in balancing motility, biofilm formation, and host interaction in *P. mirabilis*. Loss of *umoB* function promotes a shift toward a biofilm-associated, colonization-oriented phenotype characterized by increased surface attachment and reduced invasiveness. This suggests that *umoB* may be an important regulatory element in the transition between planktonic, invasive, and sessile lifestyles. Understanding this regulatory mechanism could contribute to the development of strategies targeting biofilm-associated infections in CAUTIs.



## P11.11 - SAME BACTERIA, DIFFERENT BIOFILM: EXPERIMENTAL INFLUENCES ON STAPHYLOCOCCUS AUREUS BIOFILMS

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**Background & Aims:** *Staphylococcus aureus* (*S. aureus*) is the leading cause of cardiovascular biofilm infections. *S. aureus* biofilms can cause a broad spectrum of infections, ranging from localized to life-threatening systemic infections, and are associated with prolonged hospitalization, the need for more aggressive treatment, and increased mortality rates. Understanding the factors that influence biofilm formation is critical for developing targeted therapeutic strategies. This study investigated how different experimental environmental conditions affect *S. aureus* biofilm development.

**Methods:** Clinically isolated *S. aureus* strains 25268 and 50825, along with the commonly used laboratory strain *S. aureus* Newman, were cultured in Tryptic Soy Broth, Iscove's Modified Dulbecco's Medium, or 100% human plasma. Bacterial growth and metabolic activity were measured over 24h at 37°C using optical density measurements and isothermal microcalorimetry. Biofilm development was visualized and tracked using confocal laser scanning microscopy. For live time-lapse imaging, two bacterial labelling strategies were used: GFP-based transformation and MycoLight Red JJ94™. Fibrin was visualized using fibrinogen conjugate Alexa Fluor 647. For end point imaging, SYTO9 (live bacteria) and propidium iodide (dead bacteria) were used.

**Results:** Bacterial growth and metabolic activity were medium-dependent, with MycoLight reducing maximum metabolic activity and bacterial growth in a strain- and medium-dependent manner. In TSB, neither MycoLight nor GFP labeling affected growth. In IMDM, all strains stained with MycoLight showed a significantly decreased metabolic activity compared to unstained controls. Biofilms also differed visually depending on the growth environment. Fibrin-rich biofilms only developed in human plasma, unstained 50825 and 25268 formed dense fibrin networks after 24 h. When MycoLight was added at  $t_0$  to visualize the bacteria, fibrin formation in 25268 decreased by 59.1%, while 50825 showed 99.0% reduction after 24h. In contrast, GFP-labeled 50825 still developed a fibrin network, a 27.3% decrease compared to unlabeled control.

**Conclusions:** *S. aureus* biofilm development is influenced by both growth media and live-cell labelling methods; these factors can alter bacterial behavior even within a single species, particularly under host-like conditions. The same bacteria form different biofilms depending on experimental conditions, making careful selection and context-specific validation essential for accurate interpretation of experimental data.



## P11.12 - INFECTIVE ENDOCARDITIS BIOFILMS: TARGETING THE FOUNDATION

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**Background & Aims:** Infective endocarditis (IE) is a life-threatening bacterial infection affecting the heart valves and surrounding tissue. Standard prolonged antibiotic therapy often fails against IE biofilms, necessitating invasive valve replacement surgery. Within the cardiovascular system, bacteria interact with blood components to facilitate biofilm formation. Visualizing the structural components is critical for identifying the foundation that must be targeted to overcome these biofilms.

**Methods:** Five clinical IE bacterial isolates, *Enterococcus faecalis*, *Streptococcus gordonii*, *Staphylococcus ugdunensis*, and two *Staphylococcus aureus* (most common infecting microbe), were grown on porcine heart valves in 100% human plasma. Biofilm development was visualized over time using three-dimensional confocal microscopy, and metabolic activity monitored using isothermal microcalorimetry. *S. aureus* biofilms were treated with flucloxacillin with or without prior treatment with matrix-degrading enzymes. Biofilm degradation and clearance were quantified using live confocal microscopy. Bacterial viability after treatment was assessed using isothermal microcalorimetry.

**Results:** Different biofilm phenotypes were observed, with strain-specific fibrin(ogen) binding and aggregate formation. *S. aureus* fibrin formation began at 1.8 h (SEM 0.3), with most fibrin developing within 6 hours. Enzymes reduced biofilm mass by 65% (range 45-81%) within 30 min, and 67% (range 45-87%) within 15 min for higher doses. Less than 20 bacteria per field-of-view remained after saline rinse. Metabolic activity decreased upon enzyme treatment with peaks at 13.39  $\mu$ W (SEM 10.20) in plasma, and 6.92  $\mu$ W (SEM 3.45) in plasma with 0.25 mg/mL flucloxacillin, with no detectable metabolic activity at 9.98 h (SEM 2.85) under flucloxacillin exposure. Untreated biofilms peaked at 34.00  $\mu$ W (SEM 8.09) and 30.75  $\mu$ W (SEM 8.09), with metabolic activity remaining detectable at 16 hours independent of flucloxacillin, while biofilm-detached bacteria peaked at 39.08  $\mu$ W (SEM 2.65) and 2.96 (SEM 1.20) and no metabolic activity was measured at 8.63 h (SEM 2.96) in the presence of flucloxacillin. CFU counts and confocal microscopy confirmed reduced bacterial survival following treatment and antibiotic exposure.

**Conclusions:** Clinical IE strains showed different biofilm architectures and fibrin(ogen) incorporation. Enzymes targeting the biofilm-matrix present a promising strategy for biofilm removal by degrading plasma-grown *S. aureus* biofilms within 15 minutes, thereby facilitating bacterial removal and enhancing susceptibility to flucloxacillin.



### **P11.13 - CANDIDA ALBICANS PULMONARY INFECTION IN WILDTYPE AND CYSTIC FIBROSIS-LIKE MOUSE MODELS HIGHLIGHT LIPID DYSREGULATION AS A PATHOGENIC MECHANISM**

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**Background & Aims:** Fungal infections in the lungs of people with Cystic Fibrosis (pwCF) occur with higher frequency due, in part, to repeated courses of antibiotic treatments to address other bacterial pathogens. The opportunistic pathogen *Candida albicans* (Ca) is a dimorphic fungal species that is routinely found in the lungs of pwCF and outgrowths typically require treatment with anti-fungals. To date, there is no adequate model of Ca outgrowth in a mouse lung phenocopy model of pwCF. Although CF transmembrane conductance regulator KO mice do not recapitulate features of the CF lung, Scnn1b-transgenic ( $\beta$ ENaC) mice overexpressing a sodium channel display defective mucociliary clearance, dehydrated viscous mucus, and airway neutrophilia.

**Methods:** We used wildtype and  $\beta$ ENaC mice, both on a BALB/c background, to model a Ca outgrowth and establish the conditions necessary for tissue invasion and dissemination. We induced neutropenia in half of the mice by administering cyclophosphamide at 3 and 1 day prior to challenge. Mice received intranasal instillation of Ca isolate 529L, and tissues were collected at 1 day post-infection. Ca burdens in the kidneys evaluated dissemination. Lung lipidomics were assessed by mass spectrometry imaging of Norharmane-coated tissue sections performed in negative ion mode using a Bruker timsTOF Flex.

**Results:** We found only neutropenic mice, regardless of genotype, showed Ca dissemination to the kidneys following intranasal challenge. The early onset impact of Ca on the tissue was greater in WT lungs than  $\beta$ ENaC, with the WT lungs showing severe hallmarks of pathology throughout the lungs. Further, the WT mice had higher Ca burdens in the kidneys at 24 hours post-infection than the  $\beta$ ENaC lungs. We investigated the tissue and found evidence of a morphological transition in Ca growth in the neutropenic lungs to the hyphal form that could not be identified in the non-neutropenic lungs. Finally, we assayed the model system for lung neutrophil-associated lipids and identified new, Ca-outgrowth associated lipid signatures across the WT and  $\beta$ ENaC lungs.

**Conclusions:** Together, these data are the first report of a Ca-outgrowth model system in a CF-like mouse lung and establish a unique lipid fingerprint of the lung during invasion and dissemination.



## P11.14 - ANTIBIOTIC SUSCEPTIBILITY AND PHAGE RESPONSE OF BIOFILM-PRODUCING ENTEROCOCCUS FAECALIS

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**Background & Aims:** *Enterococcus faecalis* is an important opportunistic pathogen responsible for persistent healthcare- and community-acquired infections, largely driven by its intrinsic resistance to many commonly used antibiotics and its strong biofilm-forming capacity. Biofilm formation facilitates attachment to surfaces and host tissues and protects bacteria from immune defenses and antimicrobial agents, making infections particularly in urological settings difficult to eradicate with antibiotics alone. Therefore, screening bacteriophages active against biofilm-producing isolates is essential, as phage therapy represents a promising alternative or adjunct strategy for disrupting biofilms and overcoming antimicrobial resistance. This study investigated biofilm production and bacteriophage susceptibility among clinical isolates

**Methods:** A total of 112 non-duplicate isolates were recovered from genito-urinary clinical specimens and identified using standard microbiological methods. Biofilm-forming ability was assessed phenotypically using a microtiter plate assay. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute guidelines using ampicillin, penicillin, ciprofloxacin, vancomycin, teicoplanin, high-level gentamicin, erythromycin, linezolid, nitrofurantoin, and tetracycline. Phage susceptibility was evaluated using two lytic *E. faecalis*-specific bacteriophages, LilEfef\_55 [GenBank: PQ433352] and EfeBG\_143 [GenBank: PQ433353], by spot assay and efficiency of plating.

**Results:** Overall, 74.3% of strains were susceptible to at least one bacteriophage, and 28.6% were susceptible to both, whereas 25.7% were resistant to both phages. Biofilm analysis showed that 31.1% of isolates were strong biofilm producers, 37.5% were moderate producers, and 31.4% were weak or non-biofilm formers. Strong biofilm-forming isolates demonstrated higher phage resistance, with 67.4% resistant to both phages compared to moderate and weak biofilm producers. Reduced phage activity was particularly evident in solid-medium assays.

**Conclusions:** These findings reveal variability in biofilm formation and phage susceptibility among clinical isolates and underscore the need of further systemic studies and routine screening of *Enterococcus faecalis* isolates for improving the selection of highly active therapeutic bacteriophages.



## P12.1 - NATURAL DEEP EUTECTIC SOLVENTS AS ALTERNATIVE AGENTS FOR BIOFILM CONTROL.

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**Background & Aims:** Biofilms are structured microbial communities embedded in an extracellular polymeric substance (EPS) matrix that confers strong resistance to antimicrobials and host defences, resulting in persistent infections and industrial contamination. Natural deep eutectic solvents (NADES) have emerged as sustainable “green” antimicrobials with the potential to disrupt biofilm architecture by solubilizing EPS components. The objective of this work was to investigate how three chemically distinct NADES: choline chloride–lactic acid (CCLA), choline chloride–urea (CCU), and choline chloride–xylitol (CCX) differ in their ability to inhibit bacterial growth and disrupt biofilm structure in Gram-negative and Gram-positive model organisms.

**Methods:** NADES were synthesized using a thermal method and characterized by FT-IR, <sup>1</sup>H NMR, and viscosity analysis. Antimicrobial activity was assessed against *Pseudomonas fluorescens* WCS365 and *Staphylococcus epidermidis* ATCC 35984. Minimum inhibitory concentrations (MICs) were determined by broth microdilution. Growth kinetics at sub-MIC levels were monitored over 24 h and analysed using area under the curve (AUC). Biofilm inhibition (MBIC) and eradication (MBEC) were evaluated using crystal violet assays. Structural effects were visualized by confocal laser scanning microscopy (CLSM) following triple staining for live cells, dead cells, and EPS polysaccharides.

**Results:** *P. fluorescens* was more susceptible to NADES than *S. epidermidis*. CCLA showed the highest planktonic potency (MIC 2.5–5%), followed by CCU (10–20%) and CCX (20–25%). High sub-MIC concentrations significantly delayed growth and reduced biomass accumulation ( $p < 0.0001$ ), while low sub-MIC levels had no effect. CCLA strongly inhibited initial biofilm formation by ~85% in *P. fluorescens* and ~70% in *S. epidermidis*. CCU and CCX were effective mainly at higher concentrations. For mature biofilms, CCLA achieved up to 67% biomass reduction, while CCU showed moderate eradication (~42%). CLSM demonstrated pronounced EPS degradation and microcolony disruption after CCLA treatment. COMSTAT quantification confirmed significant reductions in EPS and live-cell biovolume relative to the control.

**Conclusions:** This study demonstrates that NADES exhibit pronounced, composition-dependent antibiofilm activity. All tested formulations showed measurable efficacy in inhibiting bacterial growth and disrupting biofilm architecture, while CCLA consistently displayed the highest overall activity across both Gram-negative and Gram-positive models. Collectively, these findings support NADES as promising, green alternatives to conventional disinfectants for the management of persistent biofilm-associated contamination.



## P12.2 - DEVELOPMENT OF A BIOFILM DRAIN MODEL AND ITS SUSCEPTIBILITY TO TREATMENT WITH SODIUM TROCLOSENE

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1 - Univeristy College Dublin

**Background & Aims:** Biofilms formed within hospital drains contain pathogenic bacteria and can pose a risk of infection to both patients and staff when water containing biofilm bacteria is splashed onto nearby surfaces and unintentionally transmitted. To reduce the risk of infections, the drain biofilm needs to be disrupted and disinfected. Previous studies have demonstrated that sodium troclosene (NaDCC) can effectively disrupt and kill bacteria within biofilms. NaDCC tablets release hypochlorous acid, a form of chlorine, when dissolved in water which releases chlorine for longer and over a larger pH range than sodium hypochlorite. As such, it is widely used as a disinfectant for swimming pools and in the food industry. The aim of this study was to develop a robust laboratory drain model to test the effectiveness of NaDCC against drain biofilms.

**Methods:** A drain model using aspirator bottles and a peristaltic pump was established to mimic the sink drain and trap. Following the formation of a drain biofilm by *Pseudomonas fluorescens* and *Staphylococcus epidermidis*, the effectiveness of NaDCC to disinfect viable biofilms was assessed by CFU and confocal microscopy.

**Results:** A 4-minute contact time at 1,000ppm of NaDCC resulted in a 5 log-fold reduction of viable bacteria. Analysis of biofilms by confocal microscopy following treatment with NaDCC showed biofilm structure and composition were disrupted.

**Conclusions:** The biofilm drain model is a representative model to test disinfectants against sink and drain biofilms. NaDCC is a promising candidate for disinfection and removal of drain biofilms.



### P12.3 - INVESTIGATING THE ANTIBIOFILM ACTIVITY OF FREE AND ENCAPSULATED POLYPHENOLS IN STATIC AND DYNAMIC CONDITIONS

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**Background & Aims:** Biofilm-associated infections remain highly refractory to antibiotic treatment, representing a persistent clinical and economic burden. Polyphenol-rich natural extracts have emerged as promising antibiofilm agents, yet their efficacy is often limited by instability and poor bioavailability. This study investigates the antibiofilm activity of green tea and propolis extracts, administered in free form or encapsulated within polymeric nanoparticles, as alternative strategies to inhibit bacterial growth and biofilm formation.

**Methods:** Growth kinetics of clinically relevant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were monitored over 24 hours by optical density measurements. Surface colonization and biofilm formation were evaluated under physiological relevant shear stress using PDMS microfluidic channels couples with phase-contrast and fluorescent microscopy. The inhibitory effect of green tea (GT) and propolis (PP) extracts were investigated either directly in the culture medium or encapsulated within nanoparticles constituted by a matrix of the self- surfactant polyester, polyhydroxybutirate-co-hydroxyexanoate (PHBHHx).

**Results:** Both free extracts and extract-loaded nanoparticles significantly inhibited bacterial growth. Notably, encapsulated formulations exhibited enhanced antibiofilm activity despite lower polyphenol concentrations. Under dynamic flow conditions, treatments markedly reduced surface coverage and strongly suppressed biofilm formation, indicating a synergistic contribution of the polyphenols and the nanoparticle carrier.

**Conclusions:** These findings highlight polyphenol-loaded self-surfactant polyester nanoparticles as a potent, non-antibiotic approach for limiting biofilm development, with clear potential for the prevention and management of biofilm- associated infections.



## P12.4 - DEVELOPING A MODEL SYSTEM TO TEST EFFICACY OF ANTIMICROBIAL MATERIALS FOR THE CONTROL OF BIOFILM- FORMING CYANOBACTERIA

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1 - Student; 2 - Researcher

**Background & Aims:** Worldwide, over two billion people consume water potentially contaminated with pathogenic bacteria, including biofilm-forming cyanobacteria and the toxic compounds they produce. Due to many communities' reliance on off-grid solutions, such as wells or open water sources, it has been proposed to develop novel water treatment technologies applicable to localised environments lacking large-scale infrastructure; technologies that may include antimicrobial materials. However, to test the efficacy of antimicrobial materials, reliable and standardised methods are required, yet these are currently lacking. Therefore, this project's aim is to develop methodologies for testing the efficacy of antimicrobial materials designed to control biofilm- forming cyanobacteria.

**Methods:** Preliminary work focused on developing a reproducible model system to consistently test the efficacy of antimicrobial materials in controlling the biofilm-forming cyanobacteria *Nostoc*, *Oscillatoria*, and *Gloeocapsa spp.* Determining biomass and cell death in cyanobacterial biofilms can be challenging due to their filamentous and/or colonial morphology. Several methods were therefore tested to accurately determine biomass and live/dead ratios. These included homogenisation techniques to improve uniformity of filamentous cyanobacterial cultures (*Nostoc* and *Oscillatoria spp.*) to facilitate quantification, as well as the optimisation of fluorescence staining protocols to enable live/dead staining and overcome chlorophyll autofluorescence. Methods were then tested using bismuth oxide (BiOx) as a visible-light-activated antimicrobial material, with efficacy determined by reductions in optical density, and changes in cell viability via fluorescence imaging using a ZEISS upright Airyscan confocal microscope. Data from BiOx-treated surfaces were compared with those from control surfaces.

**Results:** Results include live/dead fluorescence microscopy images illustrating cell viability and death in *Nostoc*, *Oscillatoria*, and *Gloeocapsa spp.* Quantitative results include optical density measurements and corresponding live/dead cell counts, demonstrating changes in cyanobacterial growth and cellular inactivation across treated and control surfaces.

**Conclusions:** Due to their colonial and filamentous morphology, the routine and accurate determination of biomass and cell death in cyanobacteria can be difficult. Here, methods have been developed and optimised for the measurement of optical density and cell viability. These approaches facilitate the testing of antimicrobial materials against cyanobacterial biofilms and form the basis for the development of a standardised operating procedure for the testing of antimicrobial materials for biofilm control.



## P12.6 - ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF EBSELEN-BASED INHIBITORS OF BACTERIAL UREASES

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**Background & Aims:** Small-molecule inhibitors of microbial virulence factors are among the core strategies of combating antimicrobial resistance. Exemplary targets may include urease (allowing the use of urea as a nitrogen source and neutralizing the environment's acidic pH) or thioredoxin reductase, which reduces oxidative stress. Ebselen is a promising lead structure for the design of such molecules, and it is being studied for its biological properties, most of which result from its high reactivity with thiols. The purpose of this work was to evaluate the potential of a range of ebselen derivatives to inhibit the aforementioned virulence factors in species such as *Proteus mirabilis* and *Helicobacter pylori*, and to confirm their antimicrobial activity against biofilms.

**Methods:** Microbial urease was purified using a multistep chromatographic procedure utilizing ÄKTA Pure system. Antiureolytic properties of the compounds were studied with the cresol red pH assay and the indophenol assay of ammonia concentration. Antimicrobial activity of the confirmed urease inhibitors was assessed using the serial dilution method in Mueller-Hinton broth, in accordance with CLSI standards, and the Drop Plate method. Gravimetric measurements and microfluidic techniques using the BioFlux 1000 were employed to assess the antibiofilm activity of the most promising derivatives.

**Results:** Studied derivatives were confirmed as inhibitors of microbial urease in low nanomolar concentrations ( $K_i = 16.7$  pM for the most active one). The most active antimicrobial of them had a MIC = 12.5  $\mu$ M towards the planktonic cells of *H. pylori*. The same dose of the compound allowed to lower the amount of biofilm formed in microfluidic conditions by 43% in comparison to the positive control. The viability of the remaining cells, measured by a fluorescent assay, decreased by 83.5%.

**Conclusions:** Organoselenium compounds were confirmed to be not only highly active inhibitors of urease but also potent antimicrobials towards both planktonic and biofilm form of pathogenic microorganisms. Covalent binding of cysteine was proved to be significant for the urease inhibition. The mechanism of the antimicrobial activity goes beyond the control of urease and includes the generation of excessive oxidative stress.



## P12.7 - A HYDROGEL-BASED MICRONEEDLE SYSTEM TO OVERCOME ANTIBIOTIC-RESISTANT WOUND BIOFILMS

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**Background & Aims:** Antimicrobial resistance (AMR) poses a significant challenge to the treatment of wound infections, particularly when dense bacterial aggregates (BAs) form deeply embedded within tissue. These aggregates display increased antibiotic tolerance and evade host immune responses, rendering conventional topical or systemic therapies insufficient. Effective treatment strategies therefore require delivery systems capable of penetrating deep tissue, disrupting BA-associated biofilms, and releasing antimicrobial agents directly at the site of persistent infection. This work aims to develop responsive hydrogel microneedles for localized, deep tissue delivery of biofilm matrix-degrading catalytic enzymes together with bactericidal agents to target BAs and combat wound infections.

**Methods:** Microneedle arrays are fabricated via micromolding in PDMS molds using a degradable hydrogel matrix engineered to create minimally invasive, mechanically robust, and responsive drug delivery systems. Matrix-disrupting enzymes are encapsulated within protective carriers to preserve and improve catalytic activity. These carriers are characterized using physiochemical methods including FTIR, TGA, DLS, and PXRD. In parallel, alternative bactericidal components are incorporated into the microneedle patches to enable localized multimodal antimicrobial activity. Additionally, *in vitro* experiments evaluate hydrogel degradation, biofilm eradication, antimicrobial efficacy, and cytocompatibility, using image-based, biochemical, and molecular assays (CFU quantification, CLSM imaging, spectrophotometry, qPCR).

**Results:** Preliminary findings indicate successful fabrication of hydrogel microneedles with adequate mechanical strength and controlled degradation and release for effective tissue penetration and localized drug distribution. Carrier-stabilized catalytic enzymes were successfully encapsulated and retained catalytic activity demonstrated by effective biofilm-matrix disruption, alongside synergistic antibacterial activity from co-delivered antimicrobial agents. Furthermore, cytocompatibility was maintained and assays showed accelerated cell proliferation and migration relative to controls.

**Conclusions:** This work aims to establish a multifunctional microneedle platform capable of co-delivering biofilm-disrupting agents together with antibacterial agents into wound regions where bacterial aggregates persist. By combining minimally invasive local delivery, responsive hydrogels, and synergistic activity of biofilm-disrupting and bactericidal agents, this approach offers a targeted antimicrobial strategy for treating persistent, antibiotic-resistant wound infections.



## P12.8 - EFFECTS OF L7CF197 ON BIOFILMS FORMED BY CANDIDOZYMA AURIS

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**Background & Aims:** *Candidozyma auris* is becoming a serious public health problem due to outbreaks in healthcare settings and its multidrug resistance to available antifungal drugs. The search for molecules with antifungal potential for the treatment of infections caused by *C. auris* and for biofilm eradication, from both natural and synthetic sources, has expanded. Indole derivatives are molecules with potent activity against *Candida* species. The aim of this study was to evaluate the activity of L7CF197 against mature biofilms produced by *C. auris*.

**Methods:** L7CF197 was tested at concentrations of 0.2–600 µg/mL to assess activity against planktonic cells. A volume of 100 µL of the compound was added to each well of the microplates along with 100 µL of a standardized inoculum at a density of  $4 \times 10^3$  cells/mL, and the plates were incubated for 48 hours. Subcultures were performed on Sabouraud dextrose agar plates to determine the minimum fungicidal concentration. To assess activity against biofilms, an inoculum containing  $1 \times 10^6$  cells/mL in RPMI-1640 was prepared, and 200 µL was added to each well of the microplates. The plates were incubated for 96 hours. After the incubation period, dilutions containing 75, 150, 300, and 600 µg/mL were added to the wells. Amphotericin B and micafungin were used as controls. The plates were incubated for an additional 48 hours, after which viability was determined using the XTT assay (2,3-Bis (2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide).

**Results:** L7CF 197 exhibited minimum inhibitory concentration and fungicidal activity at 18.25 µg/mL. Micafungin was not able to inhibit mature biofilm. Amphotericin B inhibited 50% of the mature biofilm at concentrations of 4 and 2 µg/mL. L7CF 197 exhibited significant activity against the mature biofilm, with post-treatment viabilities of 6%, 6.7%, 7.5%, and 17% at concentrations of 600, 300, 150, and 75 µg/mL, respectively.

**Conclusions:** L7CF197 exhibits potent activity against mature biofilms formed by *C. auris* and may represent an alternative for developing drugs or disinfectants to eradicate this yeast.



## P12.9 - EXPLORING ANTIFUNGAL ACTIVITY OF POLYPHENOLIC AND NITROFURAN COMPOUNDS ON *TRICHOPHYTON RUBRUM* PLANKTONIC AND BIOFILM FORMS: A TUG OF WAR AGAINST DRUG RESISTANCE

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**Background & Aims:** Dermatophytosis, most commonly caused by *Trichophyton rubrum*, is a highly prevalent and neglected fungal infection in which biofilm formation enhances adhesion, persistence, recurrence, and resistance to antifungal therapies. Current treatments are limited by low drug bioavailability, poor specificity, and increasing resistance, highlighting the need for new therapeutic strategies, particularly those targeting biofilms. This study investigated the antifungal activity of multiple compounds against both planktonic cells and biofilms and evaluated cytotoxicity as an indicator of therapeutic safety, with the aim of informing the development of more effective and safer treatment approaches.

**Methods:** Several polyphenolic and nitrofurantoin compounds were quantified for antifungal activity using the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) assays, and Antibiofilm activity against *T. rubrum* ATCC MYA 4438. The MIC was conducted according to document M38-A2 of the Clinical and Laboratory Standards Institute (CLSI). MFC was determined by incubating an aliquot of each concentration tested in the MIC experiment on Sabouraud Dextrose Agar at 37°C. *T. rubrum* biofilm was formed in RPMI medium over 72 h. Subsequently, antibiofilm experiments were conducted by treating initial and mature biofilms with the compounds. The resulting biofilm's biomass and metabolic activity were gauged through crystal violet and tetrazolium salt reduction (XTT®), respectively. The cytotoxicity of the most promising compound was also assessed in epithelial cells using the resazurin assay.

**Results:** A robust *T. rubrum* biofilm formed, characterized by high metabolic activity and biomass production, maturing after 72 hours. Among the tested compounds, 10 demonstrated significant fungistatic activity ( $\leq 15,62 \mu\text{g/mL}$ ), whereas 2-hydroxychalcone, 3'-hydroxychalcone, 4'-hydroxychalcone, D-1AC, and the nitrofurantoin L7CF166 exhibited the most significant fungicidal activity ( $\leq 15,62 \mu\text{g/mL}$ ), all of which showed considerable antibiofilm activity, comparable to that of the control antifungal drugs used. In general, the compounds presented low cytotoxicity at therapeutic concentrations, with at least 70% cell viability, suggesting their safety.

**Conclusions:** This study may indicate promising new options for the treatment of dermatophytosis. *T. rubrum* biofilm formation *in vitro* was characterized and subsequently treated with the selected compounds, demonstrating significant activity against *T. rubrum* in both planktonic and biofilm forms with low cytotoxicity on human cells.



## P12.10 - FUNCTIONAL FUNGAL PROTEINS AND NANOMATERIALS AS NOVEL STRATEGIES FOR BIOFILM CONTROL

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**Background & Aims:** Bacteria in biofilms are protected from environmental stressors and therefore exhibit strong tolerance to current biofilm removal strategies, such as chemical disinfectants. The aim of this study was to systematically evaluate promising and innovative biofilm removal approaches for persistent *Listeria innocua* and *Listeria monocytogenes* biofilms, specifically methods based on nanomaterials and functional fungal proteins. Functional fungal proteins from the group of glycan-binding proteins, lectins, possess unique properties and high stability, which makes them promising candidates for targeting bacterial surface structures and inter-bacterial communication, potentially impairing biofilm development or promoting its removal. Anisotropic magnetic nanoparticles (AMPs) composed of iron oxide facilitate magneto-mechanical disruption of biofilms when exposed to a rotating magnetic field at relatively low speeds. Silver nanoparticles, known for their antibacterial effects, are used as chemical means for biofilm removal. We primarily investigated how the structural properties of AMPs, such as surface roughness and size, influenced biofilm removal, and secondly, how magneto-mechanical disruption combined with chemical and biochemical approaches could achieve synergy and improve biofilm removal.

**Methods:** To track the responses of bacteria in biofilm to selected antibiofilm strategies, we used classical colony-forming unit quantification to assess viability, measured growth curves, performed bacteria-lectin agglutination assays, and used scanning electron microscopy for visualization or fluorescence microscopy, which was indicative for effect of fungal proteins.

**Results:** Our findings reinforce the importance of novel and combined methods for biofilm removal. Fungal proteins significantly impaired biofilm development by modulating interactions between bacteria and/or between bacteria and surfaces, which could be used in the future to reduce surface contamination by both species of *Listeria*. AMP surface roughness did not influence biofilm removal, whereas particle size had a pronounced impact. Silver nanoparticles grafted onto the most effective AMPs produced a synergistic mechanical-chemical effect, enhancing *L. innocua* biofilm disruption.

**Conclusions:** Our results indicate that novel biofilm removal methods, such as the use of functional fungal proteins and magnetically triggered hybrid-nanomaterials, represent a promising approach for achieving reliable and robust biofilm removal from surfaces. While fungal lectins predominantly affect biofilm formation during the adhesion phase, hybrid nanomaterials can be used to target mature biofilms.



## P12.11 - COMBATING IMPLANT-RELATED ORTHOPAEDIC INFECTIONS WITH THERAPEUTIC ULTRASOUND AND SHOCKWAVES

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**Background & Aims:** Orthopaedic implant-associated infections are driven by biofilm-forming bacteria and remain difficult to eradicate due to poor antibiotic penetration and immune evasion. These infections impose substantial clinical and economic burdens, often requiring revision surgery. Mechanical biofilm disruption via cavitation - the formation and collapse of microscopic gas bubbles - generated by therapeutic ultrasound (US) or shockwaves (SW) represents a minimally invasive adjunct to antimicrobial treatment but has not been evaluated systematically in clinically relevant orthopaedic models. This study compares US and SW against *Staphylococcus aureus* biofilms on implant materials, both with and without antibiotics.

**Methods:** Osteomyelitis-derived *S. aureus* strains were cultured on ~12-mm discs of medical-grade stainless steel, titanium, and hydroxyapatite in synthetic wound fluid to mimic implant and bone surfaces. Samples were exposed to US at 0.95 MHz, peak negative pressure (PNP) 2.5 MPa, 500 Hz pulse repetition frequency (PRF), 10% duty cycle, and 10 s per location across ten positions (200 J incident acoustic energy), in the presence of protein-based cavitation nuclei. SW treatments were applied without nuclei and included stationary exposures (50 SW at 3.5 MPa PNP, 0.5 Hz PRF; 70 mJ) or scanning exposures (six positions, one SW each at 3.5 MPa; 5 mJ). Bubble activity was characterised by passive cavitation detection, which measured acoustic emissions. Biofilm surface coverage was assessed by fluorescence imaging and biofilm burden by colony-forming unit (CFU) counts.

**Results:** All standalone US/SW treatments significantly reduced biofilm burden on the discs, measured as log<sub>10</sub> reductions in CFU recovered from the discs, but were not bactericidal. Stationary SW achieved a 3.8-log<sub>10</sub> CFU reduction (95% CI: 3.1-4.5), scanning SW a 2.5-log<sub>10</sub> reduction (95% CI: 1.8-3.2), and scanning US a 1.2-log<sub>10</sub> reduction (95% CI: 0.8-1.6), spatially mirroring the US beam FWHM. Bacteria, released into the supernatant from disrupted biofilms and exposed to vancomycin and rifampicin, exhibited minimum inhibitory concentrations (MICs) of 1 µg/mL for vancomycin and 7.5 ng/mL for rifampicin, matching planktonic MICs.

**Conclusions:** These data demonstrate that US- and SW-driven cavitation disrupts implant-associated biofilms and restores antibiotic susceptibility of *S. aureus*, supporting their use as adjuncts to enhance antibiotic efficacy in orthopaedic implant-associated infections, with potential implications for AMR.



## P12.12 - ADVANCING BIOFILM CONTROL: FUNCTIONALIZED-NANOMATERIALS FOR LIGHT-TRIGGERED ANTIBIOFILM ACTION

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**Background & Aims:** *Staphylococcus aureus* biofilms cause urinary and wound infections, with MRSA strains resistant to antibiotics posing severe health risks. Antimicrobial photodynamic inactivation is a promising alternative. We aimed to design photoactive nanocomposites with potent antibiofilm activity and confirm their mechanism of action.

**Methods:** Standard strain *S. aureus* CCM 3953, and two clinical MRSA isolates (*S. aureus* S61 and L12) were used. Nanocomposites based on poly(urethane) (PU) and saponite particles modified with poly(diallyldimethylammonium) chloride (1.5 mmol/g) and photosensitizers – Erythrosine B (EryB), Eosin Y (EosY), or Phloxine B (PhB) were prepared. The nanocomposite thin films on PU were irradiated with a green laser ( $\lambda = 532$  nm, 100 mW) for 2 or 10 min. The *in vivo* toxicity of individual components was evaluated using the *Galleria mellonella* model. Antibiofilm activity was assessed on 24-h biofilms and quantified by colony-forming units. The production of reactive oxygen species (ROS) before and after illumination was determined using the ROS-Glo™ H<sub>2</sub>O<sub>2</sub> Assay kit. PhB release from the nanocomposite was monitored and analysed by absorption spectroscopy.

**Results:** No toxicity of the individual components was observed in *G. mellonella* larvae over a 5-day monitoring period. Among tested materials, PhB-modified nanocomposites demonstrated the highest antibiofilm effectiveness, achieving over 2.8 log<sub>10</sub> reduction after 2 min of irradiation in 24-h biofilms formed by the strain CCM. Nanocomposites functionalized with EryB and EosY achieved 2.5 log<sub>10</sub> and 1.7 log<sub>10</sub> reductions, respectively, after 10-min irradiation, indicating that prolonged irradiation was required to reach an effect comparable to that of PhB. In MRSA clinical isolates S61 and L12, PhB-modified nanocomposites achieved 2.3 log<sub>10</sub> reduction after only 2 min of irradiation. ROS analysis revealed 15-, 17-, and 10-fold increase in irradiated samples for CCM, S61, and L12, respectively. The release kinetics of PhB from the nanocomposites followed a rate constant of  $(1.6 \pm 0.9) \cdot 10^{-2} \text{ h}^{-1}$ , with an intensive initial release during the first hours and a half-time of approximately 31 h.

**Conclusions:** These results underscore the strong potential of functionalized nanocomposites as a promising strategy for the effective control of MRSA biofilms.

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## P12.13 - CORTICOSTEROIDS INCREASED THE PRODUCTION OF QUORUM SENSING SIGNALLING MOLECULES IN A BIOFILM-GROWING CLINICAL PSEUDOMONAS AERUGINOSA ISOLATE FROM CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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**Background & Aims:** *Pseudomonas aeruginosa* (PA) frequently colonises the lungs of individuals with Chronic Obstructive Pulmonary Disease (COPD), for which corticosteroids are the key treatment to reduce airway inflammation. Corticosteroids promote biofilm formation in PA (PMID: 40539031) via c-di-GMP regulation, contributing to the pathophysiology of COPD. Given the interconnection between c-di-GMP and Quorum Sensing (QS) in biofilm regulation we aimed to investigate if corticosteroids directly affected QS signalling molecules (QSSMs) production.

**Methods:** Biofilms of PAO1-N wt and 3 QS isogenic mutants unable to produce QSSM ( $\Delta pqsA$ ,  $\Delta lasI$ ,  $\Delta rhlI$ ) were prepared in RPMI1640 media (OD<sub>600</sub>=0.1) and treated with 1  $\mu$ M of corticosteroids (fluticasone or budesonide) or DMSO (solvent control) and imaged by confocal microscopy after 48h of incubation. For the analytical quantification of QSSM, supernatants of 24h biofilms of PAO1-N wt and clinical strain JQS-01 (COPD isolate) treated with 1 $\mu$ M fluticasone or budesonide or DMSO were filtered and QSSM were extracted and analysed by LC-ESI-MS/MS (HHQ, HQNO, PQS, C4-AHL and oxo-C12-AHL).

**Results:** While PAO1-N showed an increase of biofilm biomass in response to corticosteroid treatment, the 3 isogenic QS mutants showed no difference. No significant differences were found for the production of HHQ, HQNO, PQS, C4-AHL and oxo-C12-AHL between DMSO and corticosteroid treatment for PAO1-N at 24h. This could be related to the lower production of these molecules by PAO1-N compared with other PAO1 type strains. The clinical strain JQS-01 showed a statistically significant increased production of all alkyl-quinolones in response to both corticosteroids and a significant increase of C4-AHL in response to fluticasone, which aligns with the observed biofilm biomass increase at 24h (PMID: 40539031).

**Conclusions:** The involvement of QS in the modulation of *P. aeruginosa* biofilm formation by corticosteroids could open the door to the utilization of QS inhibitors as adjuvants for the treatment of COPD, with the aim of reducing *P. aeruginosa* biofilm formation. This is important given *P. aeruginosa* is difficult to treat due to its inherent resistance to antimicrobials and the immune system.



## P12.14 - NITROGEN-DOPED GRAPHENE POLYMERIC COMPOSITES INHIBIT SINGLE- AND MULTI-SPECIES UROPATHOGENIC BIOFILMS

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**Background & Aims:** Indwelling urinary catheters, often manufactured from silicone-based materials such as polydimethylsiloxane (PDMS), provide a favourable substrate for uropathogenic biofilm formation and are a major source of catheter-associated urinary tract infections. There is therefore a need for catheter coatings that intrinsically limit biofilm development by single- and multi-species communities. This study aimed to produce nitrogen- doped graphene nanoplatelet (N-GNP)/PDMS composites and evaluate their ability to curb mono- and polymicrobial biofilms by representative uropathogens under urine-like conditions, focusing on total cells, culturability and biofilm structure.

**Methods:** N-GNP was synthesized by melamine-assisted ball milling followed by thermal treatment and incorporated at 1 wt.% into a PDMS matrix to obtain thin composite films, which were characterized for surface morphology, roughness and hydrophobicity. Single-species and tri-species biofilms of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were grown in artificial urine medium for 24 h on N-GNP/PDMS and unmodified PDMS surfaces. Total and culturable biofilm cells were quantified by flow cytometry and colony-forming unit counts, respectively. Confocal laser scanning microscopy was used to reconstruct biofilm architecture and determine biovolume, thickness and species distribution within mixed communities.

**Results:** N-GNP/PDMS composites were rougher and more hydrophobic than bare PDMS. In single-species biofilms, N-GNP/PDMS reduced total cell numbers of *S. aureus*, *P. aeruginosa* and *K. pneumoniae* by approximately 64%, 41% and 29%, respectively, relative to PDMS; culturable *S. aureus* cells were reduced by ~50% on N- GNP/PDMS, whereas culturable *P. aeruginosa* and *K. pneumoniae* remained comparable to PDMS despite lower total cell numbers. In tri-species biofilms, N-GNP/PDMS showed an overall reduction of ~41% in total cells. Confocal analysis showed decreased biovolume and thickness on the composite surface, with the most pronounced reductions observed for *S. aureus* in both mono- and multi-species settings.

**Conclusions:** Incorporating a low fraction of N-GNP into PDMS yields a graphene-based polymeric surface that significantly inhibits single- and multi-species uropathogenic biofilms, with the strongest effect against *S. aureus*. Nitrogen-functionalized graphene/PDMS composites are therefore promising candidates for next- generation urinary catheter coatings and warrant further investigation of long-term performance, encrustation behaviour and biocompatibility.



## P12.15 - SPECIES-SPECIFIC ANTIBIOFILM ACTIVITY OF THE BACTERIOPHAGE-DERIVED ENDOLYSIN CHAPK AGAINST MRSA AND MRSE

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**Background & Aims:** Biofilms formed by methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE) are commonly associated with device-related, implant-associated, and chronic wound infections, where they contribute to persistent colonization and limited treatment efficacy. Their pronounced tolerance to conventional antibiotic regimens highlights the urgent need for alternative antimicrobial approaches. Bacteriophage-derived endolysins have attracted considerable interest due to their rapid and specific bacteriolytic activity, achieved through targeted peptidoglycan cleavage upon exogenous application. This study focuses on CHAPk, an antistaphylococcal endolysin derived from bacteriophage K, and aims to assess its antibiofilm activity against MRSA and MRSE.

**Methods:** Recombinant CHAPk was heterologously produced, and its antibacterial activity was first assessed against planktonic MRSA (clinical isolate) and MRSE (RP62A). Subsequently, 24 h mature MRSA and MRSE biofilms were established in 96-well microplates and exposed to CHAPk at concentrations ranging from 3.2 to 45.6  $\mu$ M under agitation at 37 °C. Biofilms were harvested at 0, 4, and 24 h, and biofilm-associated cells were recovered by sonication, followed by quantification of viable cells using the microdrop plating method. Sodium acetate buffer (10 mM, pH 7.0) served as the negative control, and all experiments were performed in triplicate.

**Results:** At 2.28  $\mu$ M, recombinant CHAPk reduced planktonic MRSA and MRSE cultures from an initial OD<sub>600</sub> of 0.5 to 0.1 and 0.4, respectively, within 10 min. In biofilm assays, treatment with 45.6  $\mu$ M CHAPk for 24 h resulted in an approximately 3.2-log reduction in MRSA biofilm viability, whereas MRSE biofilms exhibited only a modest reduction of approximately 0.3 log.

**Conclusions:** The results indicate that CHAPk displays pronounced antibacterial and antibiofilm activity against MRSA, while its impact on MRSE biofilms remains limited. This species-specific efficacy underscores intrinsic differences in biofilm physiology and cell wall accessibility that influence susceptibility to endolysin-mediated lysis. Collectively, these findings support the potential of CHAPk as a targeted antibiofilm agent for MRSA-associated infections and emphasize the need for tailored strategies to enhance its activity against MRSE biofilms.



## P12.16 - EVALUATION OF A LONG-LASTING DISINFECTANT FOR BIOFILM CONTROL IN HOSPITAL GREYWATER SYSTEMS

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**Background & Aims:** Hospital greywater systems, particularly sink drains and pipework, are recognised as important reservoirs for biofilm-forming microorganisms associated with healthcare-associated infections (HAIs), including *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterobacter* spp. Conventional cleaning practices may be insufficient to ensure sustained microbial control within these systems. This study aimed to evaluate the effectiveness of a long-lasting multi-surface disinfectant in reducing microbial contamination in hospital greywater infrastructure.

**Methods:** The study was conducted in a hospital environment and included washbasin drain pipes composed of flexible polyvinyl chloride (PVC), rigid PVC and stainless steel, distributed across different hospital wards and floors. The disinfectant was applied as part of routine cleaning procedures. Microbiological sampling was performed before and after application, following a standardised protocol including sample collection, preparation, incubation, optical density measurement, colony-forming unit (CFU) quantification and microorganism identification using selective and differential culture media.

**Results:** Short-term assays demonstrated a significant reduction in microbial load 15 minutes after a single application, with reductions exceeding 3 to 5 log<sub>10</sub> CFU depending on pipe material. A subsequent one- month field evaluation, involving daily application, showed sustained reductions in microbial contamination across multiple hospital zones and floors. Effective microbial control was observed even when the applied quantity was lower than the initially recommended dose. The intervention was effective against microorganisms commonly associated with biofilm formation and HAIs.

**Conclusions:** The results indicate that the evaluated disinfectant significantly reduces microbial contamination in hospital greywater systems and may contribute to improved biofilm control in these environments. Addressing greywater infrastructure may represent an important complementary strategy for infection prevention and control in healthcare settings.



## P12.17 - EFFECTS OF GALLIC ACID-BASED ANTIFOULING AGENTS TOWARD REPRESENTATIVE MARINE BACTERIA

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**Background & Aims:** Conventional antifouling coatings often rely on copper and booster biocides that affect non-target organisms and promote resistance. The European Commission has been pushing member states to work harder to find alternatives to copper-based coatings and invest in safer systems. Two synthetic derivatives, previously obtained by us from the natural polyphenol gallic acid, were shown to prevent mussel larval colonization without causing lethality, constituting a promising alternative to copper. This study evaluated their effects on representative marine bacterial models.

**Methods:** Two marine bacterial strains, tentatively identified as *Pseudomonas toyotomiensis* and *Niallia circulans*, were exposed to the persulfated and benzamide derivatives of gallic acid under planktonic and surface-associated conditions. Viable cells were quantified by colony-forming unit (CFU) enumeration. Inhibitory effect was defined as a reduction greater than 3 log<sub>10</sub> relative to untreated controls.

**Results:** Exposure of planktonic cultures to 100 mg L<sup>-1</sup> of each compound did not exceed the predefined toxicity threshold. While minor statistical differences relative to controls were detected, they did not indicate bactericidal activity. In contrast, samples exposed to the same concentration consistently showed fewer biofilm-associated cells, suggesting interference with adhesion and early biofilm development rather than reduced viability.

**Conclusions:** By limiting biofilm establishment without biocidal effects, adding to their previously identified anti-settlement effects against shell-fouling organisms, these compounds emerge as promising environmentally compatible candidates for next-generation marine coatings.



## P12.18 - ESKAPE-ING ANTIMICROBIAL RESISTANCE; 213 NM LASER LIGHT TO COMBAT SUPERBUGS

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**Background & Aims:** Antimicrobial resistance (AMR) is one of the biggest health threats facing humanity in the 21st Century. AMR carries a predicted death toll higher than cancer and an economic burden of USD\$1 trillion by 2050. There is an urgent need for new treatments that can treat antimicrobial resistant infections. One potential tool is far- UVC light. Standard UVC light (~254 nm) has been used as a surface disinfectant for decades however, its use in patients is severely limited by toxic side-effects. While standard UVC causes skin damage, nausea and eye injury within minutes of exposure, far-UVC light (200-235 nm) is reportedly safe and therefore presents a novel solution to the AMR crisis. We present 213 nm far-UVC laser light as a novel method of combatting AMR, as a prophylactic anti-biofilm treatment. We aimed to establish this by demonstrating growth inhibition of ESKAPE pathogens as well as *Candida* sp. both dry, and in suspension.

**Methods:** *E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *E. cloacae*, and *C. albicans* 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 deg C), inhibition zones were quantified using ImageJ (n≥3). *Candida* samples were treated identically but plated on Sabouraud Agar and incubated for 48 hr. 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.

**Results:** 213 nm laser light was highly effective against all tested microbes 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 bacterial count were seen at 1s of treatment (p<0.01) and a 2-log reduction (p<0.0001) was seen after 900s.

**Conclusions:** Far-UV light showed antimicrobial activity against all ESKAPE pathogens and *C. albicans* in clinically relevant timeframes. Our data suggests that 213 nm lasers hold potential as a novel tool to combat antimicrobial resistant bacteria and act as a prophylactic anti-biofilm treatment.



## P12.19 - DUAL-LAYER LIVING HYDROGEL ENABLES ON-DEMAND DELIVERY OF PHAGES AND PROBIOTICS FOR SYNERGISTIC WOUND INFECTION THERAPY

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**Background & Aims:** Wound infections caused by multidrug-resistant bacteria such as *Pseudomonas aeruginosa* are difficult to treat due to biofilm formation and limited antibiotic effectiveness. Living therapeutics, including bacteriophages and probiotics, offer complementary antibacterial effects but have individual limitations. This study aimed to develop a pathogen-responsive dual-layer hydrogel (ProΦGel) for on-demand, sequential delivery of phages and probiotics to improve wound infection therapy.

**Methods:** ProΦGel consists of an outer gelatin PEG hydrogel loaded with lytic phages and an inner alginate microbead layer encapsulating *Lactobacillus plantarum*. Phage release is triggered by enzymatic degradation of the outer layer, followed by probiotic release from the microbeads. The hydrogel was tested using in vitro antibacterial and antibiofilm assays, as well as an ex vivo human skin infection model with *P. aeruginosa*.

**Results:** ProΦGel enabled on-demand phage release in response to *P. aeruginosa*, rapidly reducing bacterial load and disrupting biofilms. Sequential probiotic delivery modulated the local microenvironment and promoted fibroblast migration, indicative of improved wound healing. In the ex vivo skin model, ProΦGel prevented biofilm formation, eradicated pre-formed biofilms, and showed tissue compatibility, outperforming treatments with phages or probiotics alone.

**Conclusions:** The dual-layer living hydrogel allowed responsive, sequential delivery of phages and probiotics, achieving synergistic bacterial killing, biofilm disruption, and support for tissue regeneration. ProΦGel represents a promising strategy for advanced wound infection therapy and highlights the potential of integrating multiple living therapeutics into a single responsive platform.



## P12.20 - EVALUATING THE DISINFECTION EFFICACY OF BDMDAC BIOCIDES IN BIOFOULING CONTROL

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**Background & Aims:** Biofilms represent a major challenge in industrial settings due to their inherent resistance to conventional antimicrobial treatments. These complex microbial communities exhibit enhanced tolerance to disinfectants, promoting persistent infections. In industry, they disturb the operation of heat exchangers and membrane systems by increasing pressure drop, reducing heat transfer rate and enhancing corrosion risks. To avoid such problems, in this work a quaternary ammonium compound named BDMDAC was selected and its antimicrobial effect was evaluated. Here we aim to examine the impact of BDMDAC on the growth and viability of *Pseudomonas fluorescens*, in established biofilms and as biofilm prevention.

**Methods:** Antibiofouling performance was evaluated through removal and prevention assays. Three different concentrations (50, 100, 200 mg/L) were evaluated as a final polishing step. The treatment effect in biofilm structural development is visualized by Optical Coherence Tomography. The bactericidal activity is assessed by CFU counting over time. The efficacy of this approach is compared to the non-treatment assays. On one hand, *P. fluorescens* biofilms were formed during 14 days after which they were exposed to BDMDAC during 4 h. On the other hand, the same strain was exposed to BDMDAC at day 0 for 4h after which the solution was replaced by fresh media and biofilm was allowed to grow for 14 days.

**Results:** Removal assays showed a decrease both in bulk and biofilm culturable cells in a dose-dependent response. Regarding biofilm structure at the mesoscale, a roughness and porosity decrease was observed, although no effect was detected in thickness. Regarding prevention assays, BDMDAC prevents initial bacteria adhesion after 4h of exposure. However, the remaining bacteria (VBNC) not detected in culturability assays were able to recolonize and form biofilm in the same extension as the control.

**Conclusions:** These approaches aim not only to inhibit biofilm formation but also to eradicate bacteria in established biofilms. BDMDAC shows a good performance in inactivating cells on established biofilms, however it was unable to remove biomass. As a prevention treatment, VBNC issues must be further explored. This study offers valuable insights into potential applications of chemical interventions in real-world contamination scenarios.



## P12.21 - IDENTIFICATION OF A BACTERIOPHAGE THAT REDUCES THE BIOFILM OF A HYPERVIRULENT KLEBSIELLA PNEUMONIAE STRAIN

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**Background & Aims:** *Klebsiella pneumoniae* (*K. pneumoniae*) is a major human and porcine infectious agent causing diseases such as pneumonia, urinary tract infections, and septicemia. Infections with hypervirulent (hv) *K. pneumoniae* strains might lead to the liver abscess syndrome in previously healthy humans. Of particular concern in nosocomial *K. pneumoniae* infections is the formation of biofilms, which contributes to resistance against current antimicrobial agents. Lytic bacteriophages are considered a promising alternative to conventional antibiotics. The aim of this project is to elucidate the effects of bacteriophages recently isolated by our group on *K. pneumoniae* biofilms *in vitro*.

**Methods:** Bacteriophages infecting porcine and human *K. pneumoniae* strains were enriched from environmental samples and purified through multiple rounds of soft-agar overlay isolation. The effects of two bacteriophages on 24-hours-old biofilms of the hv human iucA+, rmpA+ *K. pneumoniae* strain 42KC-108224-1 were investigated in 96-well plates by the crystal violet and the resazurin assay. Furthermore, combinations of phage LEJ106 with the antimicrobial peptide (AMP) DJK-6 or the antibiotic erythromycin were tested.

**Results:** Bacteriophage LEJ103 was isolated using the porcine iucA+ *K. pneumoniae* strain 17349 as a host. Bacteriophage LEJ106 was isolated with the human strain 42KC-108224-1. Imaging by transmission electron microscopy (TEM) revealed siphovirus-like particles with non-contractile tails for LEJ103. Meanwhile, TEM images of LEJ106 showed a myovirus-like particle with a contractile tail. No effect on the biofilm of strain 42KC-108224-1 was observed for bacteriophage LEJ103. A clear biofilm-reducing effect was observed for LEJ106 ( $2.5 \times 10^7$  PFU/200  $\mu$ L), which significantly reduced the biofilm biomass by 79%. These results were confirmed by the resazurin assay. However, the anti-biofilm effect of phage LEJ106 was not enhanced through combination with the AMP DJK-6 or the antibiotic erythromycin.

**Conclusions:** The bacteriophage LEJ106 showed a clear effect in reducing the biomass of a 24-hours-old biofilm of the hv *K. pneumoniae* strain 42KC-108224-1. This is promising for the development of a bacteriophage-based therapy of biofilm-related *K. pneumoniae* infections.



## P12.22 - COPPER-DECORATED MULTI-WALLED CARBON NANOTUBES FOR THE CONTROL OF MEDICAL DEVICE-ASSOCIATED BIOFILMS

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**Background & Aims:** Biofilm formation on implantable medical devices, such as urinary catheters and prosthetics, is a leading cause of persistent infections and subsequent device failure. The development of novel materials capable of preventing or disrupting bacterial biofilms is therefore crucial to address this global challenge. Carbon nanotubes have attracted growing interest in medical applications owing to their unique properties, including intrinsic antimicrobial activity and potential to be functionalized with antimicrobial agents, polymers, or metals. This study investigates the antimicrobial activity of copper-decorated multi-walled carbon nanotubes (Cu-MWCNTs) and evaluates their efficacy against pre-established *Staphylococcus aureus* biofilms.

**Methods:** Copper-decorated MWCNTs were synthesized by incipient wetness impregnation and characterized in terms of copper content, metal leaching, morphology, elemental composition, and zeta potential. The antimicrobial activity of pristine-MWCNTs (p-MWCNTs) and Cu-MWCNTs was evaluated by determining their minimal bactericidal concentrations (MBC) against *S. aureus*. Subsequently, *S. aureus* biofilms were formed on polydimethylsiloxane (PDMS) surfaces for 48 h and treated with p-MWCNTs and Cu-MWCNTs at their MBCs (5% and 3% w/v, respectively) for 6 h. Biofilms were assessed for structural integrity and cell viability using confocal microscopy, and for culturability by colony-forming unit enumeration. The effects of Cu-MWCNTs on bacterial cells were further investigated by scanning electron microscopy.

**Results:** Characterization of MWCNTs confirmed the successful incorporation of copper. Pristine MWCNTs exhibited an MBC value greater than 5 % (w/v) against *S. aureus*, while Cu-MWCNTs showed an MBC of 3 % (w/v). Confocal microscopy revealed that treatment with Cu-MWCNTs altered the spatial organization and reduced the viability of *S. aureus* biofilms. Additionally, these carbon materials significantly decreased biofilm culturability by 1.30 and 0.56 log compared with untreated biofilms or those treated with p-MWCNTs, respectively, compromising bacterial membrane integrity.

**Conclusions:** Copper deposition on MWCNTs enhanced their antimicrobial activity, enabling them to disrupt pre-established *S. aureus* biofilms. Overall, these results highlight the potential of Cu-MWCNTs as effective antimicrobial agents against medical device-associated biofilms.



## P12.23 - TARGETED DELIVERY OF A NUCLEOSIDE ANALOG VIA A LIPOGLYCOPEPTIDE CONJUGATE TO ERADICATE ANTIBIOTIC- TOLERANT PERSISTER CELLS

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**Background & Aims:** Biofilm-associated infections pose a significant clinical challenge due to their inherent antimicrobial antibiotic tolerance. Non-growing persister cells evade conventional antibiotics that target actively dividing bacteria. This results in chronic infections requiring surgical intervention. The main focus of this study is to develop a targeted drug delivery strategy to eradicate persister cells by repurposing a nucleoside analog developed for cancer treatment. We hypothesize that conjugating this analog to dalbavancin, a lipoglycopeptide with high affinity for Gram-positive bacteria, will facilitate localized delivery that maximizes activity while minimizing side effects.

**Methods:** Antimicrobial efficacy was evaluated against *Staphylococcus aureus* 29213 using a protocol where stationary phase planktonic cultures were transferred to the buffer to keep them in a non-growing state. The cultures were then exposed to the conjugate for 15 minutes, followed by a washing step to remove unbound conjugates, and 24 hours of incubation. To trigger drug release from the conjugate, N-acetylcysteine (NAC) was added during the incubation phase. Control groups included free nucleoside analog and equimolar admixtures of the unconjugated drugs. To evaluate targeting efficiency, dalbavancin was labeled with fluorescein isothiocyanate (FITC) and surface binding to planktonic bacteria and biofilms was visualized using Confocal Laser Scanning Microscopy (CLSM).

**Results:** The conjugate exhibited significant antimicrobial activity, reducing bacterial counts to the limit of detection while the non-conjugated nucleoside analogue had almost no activity. While the conjugated drug was highly effective against bacteria, it displayed no cytotoxicity. Preliminary data demonstrate that the conjugate maintains high efficacy even with short exposure times. Additionally, CLSM images further showed that the Dalbavancin targeted and bound to both planktonic cells and biofilm structures, supporting the targeting concept.

**Conclusions:** This research provides a proof-of-concept for repurposing nucleoside analogs as precision antimicrobials through lipoglycopeptide conjugation. This approach potentially offers a foundation for low-dose, targeted therapy capable of treating recalcitrant infections that involve persister cells and thereby reduces the need for surgical interventions for implant-associated infections.



## P12.24 - ANTIBIOFILM AND ANTIPATHOGENIC EFFECTS OF A HYDROETHANOLIC EXTRACT OF TANACETUM VULGARE, A PROMISING YET UNDEREXPLORED MEDICINAL PLANT

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**Background & Aims:** Microbial biofilms represent a huge challenge in clinical and environmental settings due to their high tolerance to antimicrobials, due mainly to the heterogeneous metabolic states of biofilm embedded cells, and to QS signaling networks regulating genes' expression, including virulence genes. The high level of AMR rates and incidence of biofilm associated infections determined an increasing interest in alternative/complementary anti-infectious strategies. Among these, QS inhibitors (QSIs), especially plant derived flavonoids, block signal reception and gene expression, reducing adhesion, biofilm formation and other virulence factors. The aim of this study was to evaluate the antibacterial and antipathogenic effects of *Tanacetum vulgare* (Tv.) extract against MDR (multidrug resistant) and highly virulent strains.

**Methods:** Tv. flowers and stems were collected from spontaneous flora in Romanian mountain regions. The hydroethanolic extract (70% ethanol) was analyzed by HPLC. Following initial qualitative screening, the MIC was determined using reference and 10 clinical MDR bacterial strains (Gram positive and negative nosocomial agents) from the Microbiology Laboratory collection (Faculty of Biology). Antibiotic resistance profiles were confirmed using Kirby-Bauer method. PCR assays were performed to detect resistance and virulence genes: *mecA*, *mecI*, *CIF2*, *RIF5*, *ccrC*, *SCC mecIII J1*, *kdp*, *clfA/B*, *fin*, *fnbA*, *hlg* in *Staphylococcus aureus*; *CTX-M*, *TEM*, *exoU*, *PlcH* in *Pseudomonas aeruginosa*; *OXA23*, *aph*, *ompA*, *Plc1/2* in *Acinetobacter baumannii*; *KPC*, *OXA-48*, *ompA*, *Khe* in *Klebsiella pneumoniae*. The impact of subinhibitory concentrations of Tv. extract on phenotypical resistance, on adherence to inert and cellular (HeLa cells) substrates and on hemolytic activity was subsequently assessed.

**Results:** Thirty seven components were identified in the Tv. extract, predominantly flavonoids (diosmetin, jaceosidin, luteolin, apigenin, gingerol), known for their broad pharmacological activities, including antibacterial. Tv. extract showed strong inhibitory effects, mainly against *S. aureus* and *P. aeruginosa* strains. In the presence of the extract, MDR strains regained susceptibility to several antibiotics, suggesting a synergistic effect. Adherence and hemolytic properties were significantly reduced, supporting the potential of Tv. components as QSIs.

**Conclusions:** The hydroethanolic extract of *T. vulgare* exhibited potent antibacterial and antipathogenic activities, especially attributed to its flavonoids content. These results support further research on medicinal plants as complementary tools in addressing the current antibiotic resistance crisis.



## P12.25 - CRYSTAL STRUCTURE AND ANTIBIOFILM ACTIVITY DETERMINATION OF ALTEROCIN, A SECRETED PROTEIN FROM A MARINE BACTERIUM

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**Background & Aims:** The marine bacterium *Pseudoalteromonas* sp. 3J6 secretes the antibiofilm protein alterocin (Dheilly *et al.*, 2010; Jouault *et al.*, 2020). Here, we overproduced alterocin in *Escherichia coli* and purified it, in order to experimentally determine its 3D structure and characterize its antibiofilm activities against *Pseudomonas aeruginosa*, a major threat for public health.

**Methods:** Alterocin was produced from a synthetic gene encoding a protein composed of the PelB signal peptide, a 6-Histidine tag, a TEV protease cleavage site, and alterocin. The purified recombinant protein was digested with the TEV protease releasing wildtype-like alterocin (full-length alterocin without any additional residue). The crystal structure of alterocin was determined by X-ray crystallography performed at SOLEIL synchrotron on PROXIMA-1 beamline. The antibiofilm activity of pure alterocin was evaluated against the clinical *P. aeruginosa* MUC-N1 strain under static (growth in 96-well plates followed by crystal violet staining) and dynamic conditions (growth in flow cell under a flow of medium followed by confocal laser scanning microscopy [CLSM] observation).

**Results:** Sixty-seven mg of pure alterocin was produced. Its 3D structure was determined at 1.62 Å, showing a tetramer complex with a donut-like structure. Two disulfide bridges were observed per monomer, and residues involved in hydrophobic and electrostatic interactions between subunits were identified. Static antibiofilm assays showed a dose-dependent effect with 68 and 82% inhibition of biofilm formation with 10 and 20 µM alterocin, respectively. The disruption of preformed biofilms required a higher concentration (92% at 88 µM). The results of the flow cell experiments will be presented to provide detailed data on the alterocin effects, since CLSM allows to observe the biofilm architecture.

**Conclusions:** Alterocin was overproduced, purified and crystallized. Its structure was determined, and the antibiofilm activity of pure alterocin was for the first time examined, since all previous experiments were performed on *Pseudoalteromonas* sp. 3J6 culture supernatants or partially purified extracts (Dheilly *et al.*, 2010; Jouault *et al.*, 2020). Altogether, these results are major steps towards the understanding of the alterocin mode of action and highlight alterocin as a promising candidate for the development of novel antibiofilm strategies.



## P12.26 - COMPARISON OF DIRECT AND INDIRECT PLASMA TREATMENT OF COMMON UROPATHOGENS

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**Background & Aims:** Cold atmospheric plasma (CAP), a wholly or partially ionised gas generated at or near ambient temperatures and pressure, by electrical breakdown of a carrier gas, is a novel antimicrobial strategy based on reactive oxygen and nitrogen species (RONS), with demonstrated efficacy against planktonic and biofilm bacteria. The delivery of CAP to the site of infection is an important therapeutic consideration. Treatment can be by direct exposure of the target to the plasma jet plume, or by indirect exposure, involving exposure (plasma activation) of a liquid to a plasma discharge, imbuing the liquid with plasma's antimicrobial properties via translocation of longer-lived RONS into the bulk liquid. This study aims to compare the antimicrobial activity of direct CAP treatment and plasma-activated water (PAW) treatment against type strains and clinical isolates of common uropathogens: *E. coli*, *K. pneumoniae*, *E. faecalis*, *P. mirabilis*, *S. saprophyticus*, and *P. aeruginosa*, in planktonic and biofilm states.

**Methods:** Biofilms grown on Calgary Biofilm Device MBEC pegs for 48 h were treated directly using CAP from an in-house atmospheric pressure plasma jet for up to 5 min, or immersed in PAW for up to 60 min. Chemical scavengers were used to determine the RONS responsible for the antimicrobial activity of direct CAP and PAW treatments (sodium pyruvate, tiron, haemoglobin, L-histidine, and uric acid). Finally, the long-lasting RONS, hydrogen peroxide, nitrate, and nitrite, were measured.

**Results:** Clinical isolates proved more tolerant of CAP treatment than the type strains, resisting eradication when treated directly with CAP and indirectly using PAW. Chemical scavengers showed hydrogen peroxide, superoxide, and nitric oxide were critical for the biocidal activity of direct plasma, while nitric oxide was the predominant reactive species mediating bactericidal activity in PAW. Direct CAP exposure led to rapid eradication of biofilms within 60 s, while PAW was able to inactivate biofilms within 60 min.

**Conclusions:** While direct CAP treatment is more effective at inactivating biofilms of common uropathogens, delivery to the urinary tract would be challenging. PAW treatment demonstrated promising antimicrobial activity and could be more readily delivered, e.g. as a catheter lock or bladder irrigation solution, for control of UTIs.



## P12.27 - EFFECT OF THE ACYL-HOMOSERINE LACTONASE Aii20J ON KLEBSIELLA PNEUMONIAE BIOFILM FORMATION

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**Background & Aims:** The raising of multidrug-resistant *Klebsiella pneumoniae* (KPN) strains underscores the necessity for new therapeutic strategies targeting virulence. The study of Quorum Sensing has emerged as very interesting approach for novel anti-virulence strategies, as this system regulates many virulence traits, including biofilm-formation. KPN harbours an acyl-homoserine lactone (AHL) receptor, denominated SdiA, that acts as biofilm repressor. No AHL synthase has been described in its genome, even though contradictory reports are present in the literature regarding AHL production. Previous results reported in the literature indicated that the addition of the AHL-lactonase Aii20J strongly disrupt oral biofilm formation, where the presence of AHLs is neglectable, indicating that other, yet unknown QS signals can be present. This study aimed to elucidate the effect of the AHL-lactonase Aii20J on biofilm-formation in KPN, and its relationship with SdiA.

**Methods:** KPN strains ATCC13883T and KLEB-33 (clinical), and their *sdiA*-mutants, were cultured in 15 mL LB at 200rpm for 24h. 300x-extract was obtained from 20 cultures which were acidified, dichloromethane extracted (1:1), rotavapor-dried, and resuspended in 1 mL of acetonitrile. Effect of lactonase Aii20J and 300x-extract on biofilm formation was assessed using Active Attachment biofilm-cultivation method. Filtered culture-supernatants (treated or not with lactonase Aii20J), and 300x culture-extracts were evaluated for AHL-biosensor activation using *Vibrio harveyi* JMH612 and *Escherichia coli* pSB401 in TECAN luminometer.

**Results:** Although no standard AHLs could be detected in the culture extracts, the addition of Aii20J significantly inhibited biofilm formation only in wild-type strains tested. Moreover, extracts enhanced biofilm formation but only in the wild-type strains. No effect was observed in mutant strains regarding biofilm formation treated with either Aii20J or extract. Filtered-culture supernatants and extracts activated both biosensors used. Aii20J abolished the activation of biosensors by culture supernatants, suggesting that this lactonase can attack this new signal molecule, indicating the presence of a putative new AHL-like signal-molecule. The presence of SdiA was necessary for biosensor activation, as the extracts from *sdiA*-mutants lacked the capability to activate biosensor strains, pointing at an autoinduction loop.

**Conclusions:** This study indicates the presence of a putative lactonase-sensitive signal molecule on *K. pneumoniae* cultures which could interact with the SdiA receptor, influencing biofilm formation.



## P12.28 - TARGETED BIOFILM DISRUPTION IN BONE INFECTIONS USING ULTRASOUND-ACTIVATED MICROBUBBLES FOR ENHANCED ANTIBIOTIC DELIVERY

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**Background & Aims:** Biofilm-associated infections pose a major challenge in orthopaedic surgery where methicillin-resistant *Staphylococcus aureus* (MRSA) forms antibiotic-tolerant biofilms on prosthetic materials. The extracellular polymeric substance (EPS) matrix limits antibiotic penetration and promotes tolerance, often necessitating invasive revision procedures. Therefore, better methods for improving the localised delivery and penetration of antibiotics are urgently required. This study investigates ultrasound-responsive microbubbles (MBs) as a minimally invasive method to improve treatment of biofilm-associated bone infections. Upon ultrasound activation, MBs generate localised mechanical forces that we hypothesise perturb biofilm structure and enhance antibiotic efficacy. Here we aim to exploit these effects first in a simple *in vitro* system then a more physiologically relevant *ex-vivo* human bone model.

**Methods:** MRSA biofilms were cultured for 72 hours on fibronectin-coated, acoustically compatible substrates; or on *ex-vivo* human bone tissues ( $\varnothing = 1$  cm, thickness = 0.2 cm) for seven days. Phospholipid-shelled MBs were activated using a focused ultrasound transducer (0.9 MHz, 0.5 MPa, 20% duty cycle, 100 s), alone or combined with vancomycin. Biofilm Biomass, thickness, and viability were quantified using confocal laser scanning microscopy and BiofilmQ analysis. Detached bacteria were enumerated as colony-forming units to assess bacterial dispersal.

**Results:** *In vitro* (n = 3), ultrasound-activated MBs reduced biofilm biomass from  $0.21 \pm 0.10$  to  $0.05 \pm 0.04 \mu\text{m}^3/\mu\text{m}^2$  ( $p < 0.005$ ) and thickness from  $9.8 \pm 2.5$  to  $6.0 \pm 1.8 \mu\text{m}$  ( $p < 0.05$ ). Vancomycin alone (16–64  $\mu\text{g}/\text{mL}$ ) did not significantly reduce viability compared with controls ( $80 \pm 8\%$ ,  $p > 0.05$ ), with reduction observed only at 128  $\mu\text{g}/\text{mL}$  ( $56 \pm 10\%$ ,  $p < 0.01$ ). When combined with vancomycin, ultrasound-activated MBs significantly enhanced antibiofilm efficacy; at 64  $\mu\text{g}/\text{mL}$ , viability decreased to  $25 \pm 15\%$  ( $p < 0.0005$ ), with biomass reduced to  $0.01 \pm 0.04 \mu\text{m}^3/\mu\text{m}^2$  ( $p < 0.005$ ). Combination treatment reduced detached bacterial counts by 3-4 fold compared with MB treatment alone, indicating decreased recolonisation potential. These findings are being validated in the *ex-vivo* bone model.

**Conclusions:** Ultrasound-activated MBs significantly improve vancomycin efficacy against MRSA biofilms by combining mechanical disruption with antimicrobial killing. These findings support further investigation of this minimally invasive strategy for the treatment of biofilm-associated orthopaedic infections.



## P12.29 - TARGETING WOUND BIOFILMS WITH COLD ATMOSPHERIC PLASMA VIA DIRECT TREATMENT AND DUAL-THERAPY STRATEGIES

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**Background & Aims:** Chronic wound and device-associated infections are frequently driven by polymicrobial biofilms that display marked tolerance to conventional antimicrobials. Cold atmospheric plasma (CAP) has emerged in recent times as an alternative therapy to such infections primarily through generation of reactive oxygen and nitrogen species. However, biofilm composition and the consortia of microbial species present within the community may influence treatment efficacy. This study aimed to evaluate CAP activity against clinically relevant mono-species and polymicrobial biofilms and to determine whether biofilm sensitisation using a repurposed small molecule (KHS101) could enhance CAP efficacy against tolerant *Staphylococcus aureus* biofilms.

**Methods:** Mono-species and triadic biofilms comprising *Candida albicans*, *Candida auris*, *Pseudomonas aeruginosa* and/or *S. aureus* were established in a serum-containing hydrogel-cellulose model. Biofilms were exposed to CAP (6 x argon plasma jets, 8 kV, 23.5 kHz) for up to 5 minutes. Viability was quantified using live/dead qPCR and colony counts. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) generated by CAP was measured using a fluorometric assay. For sensitisation experiments, *S. aureus* biofilms were pre-treated with KHS101 prior to CAP exposure. Morphological alterations were examined using scanning and transmission electron microscopy.

**Results:** CAP induced significant, time-dependent reductions in biofilm viability, associated with increased H<sub>2</sub>O<sub>2</sub> generation. In contrast, *S. aureus* biofilms displayed limited susceptibility to CAP in both mono-species and polymicrobial settings. Short-term KHS101 exposure alone produced modest reductions in viability without extensive structural damage. However, KHS101 pre-treatment followed by CAP resulted in marked reductions in viable *S. aureus* biofilm populations (up to ~3–4 log<sub>10</sub>), consistent with synergistic killing. Equivalent concentrations of exogenous H<sub>2</sub>O<sub>2</sub> did not replicate this enhancement, indicating that additional plasma-derived reactive species contribute to the dual-therapy effect. Microscopy revealed subtle surface perturbations following KHS101 treatment, supporting a non-lethal sensitisation mechanism.

**Conclusions:** Our CAP device demonstrates broad anti-biofilm activity, although susceptibility is species-dependent, with *S. aureus* exhibiting a strain-specific heterogeneous tolerance. Biofilm sensitisation using a repurposed small molecule restores CAP efficacy against tolerant strains enabling synergistic killing. These findings support integrated dual-therapy strategies combining plasma-derived reactive species with adjunctive sensitising agents to overcome antimicrobial tolerance in complex wound biofilms.



## P12.30 - EXTENDING ANTISENSE NUCLEIC ACID DELIVERY FROM PLANKTONIC CULTURES TO BIOFILM MODELS

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**Background & Aims:** Antisense nucleic acid mimics (NAMs) enable sequence-specific bacterial gene silencing and represent a promising alternative to conventional antibiotics. We recently demonstrated that amphiphilic dendrimers (ADs) efficiently deliver *acpP*-targeted peptide nucleic acid (PNA) into planktonic *Escherichia coli*, resulting in potent and specific antibacterial activity. To progress this platform towards clinically relevant conditions, we evaluated its performance in single- and dual-species biofilms, incorporating the gut-associated commensal *Lactiplantibacillus plantarum*.

**Methods:** Biofilms of *E. coli* alone and in combination with *L. plantarum* were established on 0.5 × 0.5 cm silicone coupons in 48-well plates and grown in Mueller-Hinton broth at 37 °C under orbital agitation (160-240 rpm). Eradication assays consisted of treating 24 h pre-formed biofilms followed by 4 h of exposure. Prevention assays involved co-incubation with AD-NAM complexes during biofilm formation, with analysis at 4 and 24 h. Complexes were tested at the concentrations previously validated in planktonic systems. Biofilm viability was determined by colony-forming unit enumeration. After treatment, biofilms were recovered from the coupons by vortexing and sonication, serially diluted, and 10 µL drops of appropriate dilutions were plated onto selective agar media. *E. coli* was enumerated on MacConkey agar and *L. plantarum* on MRS agar, enabling species-specific quantification in the dual-species biofilms.

**Results:** In established biofilms, the dendrimer exhibited intrinsic antibacterial activity, especially against *L. plantarum*. However, the addition of NAMs did not confer statistically significant synergistic inhibition against the targeted *E. coli* strain in either single- or dual-species systems. This discrepancy is consistent with the increased tolerance typically associated with biofilms. Nonetheless, in the prevention assays, short-term exposure (4 h) to AD-NAM complexes showed a trend towards enhanced inhibition of *E. coli* compared to the AD alone. These findings establish a benchmark for antisense therapy in biofilm settings and inform the following dose-optimisation steps.

**Conclusions:** This work represents a key translational step in the development of dendrimer-assisted antisense therapeutics. By extending evaluation from planktonic cultures to single- and mixed-species biofilms, we define critical parameters guiding optimisation for biofilm-associated infections. Ongoing studies focus on concentration adjustments while preserving species-specific activity within complex microbial communities.



## P12.31 - ANTIBIOFILM EFFECT OF BLACKTHORN FRUIT EXTRACT ON STAPHYLOCOCCUS AUREUS AND LISTERIA MONOCYTOGENES

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**Background & Aims:** The fruits of *Prunus spinosa* L. (commonly known as blackthorn or sloe) are well-known for their rich phytochemical composition and antimicrobial properties. Notably, the peel contains a high concentration of flavonoids, anthocyanins, phenolic acids, and tannins. Our previous research has shown that the extract from blackthorn fruit peel (BFP) exhibits strong activity against Gram-positive pathogens, such as *Listeria monocytogenes*, indicating its potential as a clean-label, plant-derived disinfectant. However, there is still limited data on its effectiveness against bacterial biofilms. Therefore, this study aimed to evaluate the antibiofilm activity of BFP extract against *Listeria monocytogenes* and *Staphylococcus aureus*, compared with their planktonic counterparts.

**Methods:** The BFP extract, obtained through ultrasound-assisted extraction, at concentrations ranging from 2 to 16 mg/mL, was added to cells of *S. aureus* (ATCC® 25923) or *L. monocytogenes* (ATCC® 15313) following growth as standing cultures, or directly applied to their 48-h biofilms formed in microtiter plate format. After 60-min treatment, a standard microbiological analysis was performed to measure viability. Flow cytometric measurements (FCM) were conducted on cells stained with SYTO-9/PI to evaluate the degree of cell damage.

**Results:** The results showed that the activity of the extract varied depending on the microbe and its growth format. A strong antimicrobial effect was observed against *L. monocytogenes* at the lowest concentration ( $p < 0.05$ ). In contrast, *S. aureus* showed less sensitivity, resulting in only a 0.47 log reduction at the highest concentration. At 16 mg/mL, a significant biofilm reduction of *L. monocytogenes* was observed, reaching 2.6 logarithmic units ( $p < 0.05$ ). Conversely, *S. aureus* biofilm was more resistant to the same extract concentration, with a maximum reduction of only 0.34 log units. At lower concentrations, no effect or a stimulatory effect was observed on the *S. aureus*. FCM confirmed that the planktonic cells suffered extensive membrane damage, with more than 90% of the population compared to biofilms, which still contained considerable viable cells.

**Conclusions:** The investigation reveals a previously unappreciated property of BFP as an effective antibiofilm agent, particularly against *L. monocytogenes*, offering new treatment options across a variety of settings, including surface disinfection. This research was funded by the National Science Centre, Poland [Grant number 2022/45/B/NZ9/00299 (OPUS-23)].



## P12.32 - LOCALIZED ANTIBIOTIC-RELEASE COATINGS: TOWARD ANTIMICROBIAL ORTHOPEDIC IMPLANTS

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**Background & Aims:** Despite advancements in aseptic techniques and improved implant materials, implant-associated infection (IAI) rates remain at 1–4% in orthopedic implants and are even higher in certain high-risk populations. These infections are associated with bacterial biofilms on implant surfaces that are highly resistant to antibiotics, making treatment difficult. To address this, a novel antibiotic-loaded, slow-release coating was developed to enable local release of antimicrobials. This study elucidates how the coating provides superior antibacterial activity compared to uncoated implants and conventional prolonged systemic antibiotic treatments. This technology will be continuously improved with the addition of antibiofilm compounds to this coating to curb biofilm formation and promote infection clearance.

**Methods:** Calgary Biofilm Device (CBD), Teflon and titanium coupons were coated with a polydopamine layer to which antibiotics were covalently bound via boric acid linker, enabling localized release. Antimicrobial activity was evaluated against biofilms formed on surface adjacent to the coated surface, modeling bacterial colonization in the peri-implant microenvironment. The coating stability and efficacy were followed for 7 days in the TSB medium. Bacterial viability assay against multiple inoculum densities ( $10^2$ - $10^7$  CFU/mL) was performed in coated CBD. The bacterial activity was analyzed by MTT staining.

**Results:** The coating was successfully deposited on clinically relevant implant substrates and demonstrated strong antibacterial activity against both planktonic cells and biofilms of clinically relevant strains, including *S. epidermidis* and *S. aureus*. Antimicrobial efficacy was retained at high bacterial inoculum ( $10^7$  CFU/mL), achieving a 2-log (99%) reduction. The coating chemistry enabled reproducible antibiotic deposition as a thin layer. This resulted in the establishment of local concentrations sufficient for effective biofilm inhibition on a nearby surface. Control experiments confirmed that polydopamine and boric acid alone did not exhibit antibacterial activity, indicating inhibition was attributable to the conjugated antibiotic. The coating maintained antimicrobial functionality after 7 days of sustained release in TSB medium.

**Conclusions:** The coating shows strong antibiofilm efficacy and high potential in preventing IAI. By enabling localized antibiotic release, this platform reduces the need for currently existing prolonged systemic antibiotic therapy. Future studies will optimize the release kinetics and validate the coating in animal models to confirm systemic safety and in vivo efficacy.



## P12.33 - PHYTOCHEMICAL QUORUM SENSING INHIBITION AS A TARGETED STRATEGY AGAINST BIOFILM-FORMING MULTIDRUG-RESISTANT BACTERIA

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**Background & Aims:** Biofilm-associated multidrug-resistant (MDR) bacteria constitute a major clinical challenge due to their intrinsic tolerance to antibiotics and persistence on biotic and abiotic surfaces. Biofilm maturation is regulated by quorum sensing (QS), a cell-density-dependent signaling network controlling virulence and structural organization. Targeting QS provides a non-bactericidal anti-virulence approach that may mitigate resistance selection. This study aimed to develop and validate a phytochemical-based QS inhibition strategy to suppress biofilm formation and virulence.

**Methods:** Pathogenic bacterial isolates with confirmed biofilm-forming ability were characterized for virulence-associated traits. Anti-QS activity of selected phytochemicals was screened using the biosensor strain *Chromobacterium violaceum*. Biofilm inhibition assays were performed on abiotic surfaces under nutrient-rich conditions. Structural deformation under phytochemical stress and viability analyses were conducted using confocal laser scanning microscopy. The Z-stack reconstruction and COMSTAT analysis quantified biofilm thickness, and surface topology. Mechanistic insights were further supported through in vitro phenotypic assays and in silico interaction studies targeting QS regulatory proteins.

**Results:** Phytochemical treatment prominently reduced biofilm formation, its biomass, and 3D architectural structure compared to untreated controls. Confocal imaging demonstrated disrupted microcolony formation and compromised matrix organization. Sub-inhibitory concentrations attenuated QS-regulated phenotypes without significantly affecting planktonic growth, indicating selective interference with bacterial communication.

**Conclusions:** The findings demonstrate that phytochemical-mediated QS inhibition effectively disrupts biofilm development in virulent pathogenic isolates. By targeting communication pathways rather than bacterial viability, this strategy offers a sustainable approach for managing persistent biofilm-associated infections. Plant-derived QS antagonists hold immense translational promise as an anti-biofilm adjuvant in different in clinical settings.



## P12.34 - BIO-INSPIRED FUNCTIONALIZED NANOCELLULOSE COATINGS FOR BIOFILM CONTROL

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**Background & Aims:** Surface-associated microbial contamination remains a major challenge in healthcare and public environments, due to biofilm formation and increased tolerance to conventional antimicrobials. Sustainable, resistance-mitigating coating strategies are urgently needed. Within the Triple-A-Coat project, we developed bio-based nanocellulose coatings functionalized with antimicrobial peptides (AMPs), including tyrocidines and gramicidin, and the biofilm inhibitor 2-aminoimidazole (2-AI). These compounds are associated with a low risk of resistance development due to their membrane-targeting and antibiofilm mechanisms, respectively. This study aimed to evaluate the antimicrobial and antibiofilm performance of the functionalized nanocellulose coatings applied to relevant substrates.

**Methods:** Nanocellulose was functionalized with antimicrobial peptides through electrostatic adsorption and with 2-AI via ion-ion interactions. The functionalized nanocellulose was applied as a thin coating on model substrates using appropriate adhesion and cohesion linkers to ensure coating stability. Antimicrobial activity was assessed against representative Gram-negative and Gram-positive bacterial strains using colony-forming unit (CFU) quantification after surface contact.

**Results:** Functionalized nanocellulose coatings exhibited significant reductions in viable bacterial counts compared to non-functionalized controls. AMP-functionalized surfaces showed significant bactericidal activity, consistent with previous studies. Coatings incorporating 2-AIs demonstrated inhibition of biofilm formation while preventing planktonic growth. The antimicrobial activity observed in single-layer coatings was retained in multi-layer coatings, which additionally provided increased mechanical stability. Control nanocellulose coatings without active agents showed no intrinsic antimicrobial effect.

**Conclusions:** Bio-inspired functionalization of nanocellulose provides an effective strategy to prevent microbial colonization and biofilm establishment on surfaces. The coatings are currently being tested in a real-world bus demonstration environment to assess both antimicrobial efficacy and durability under practical conditions. Overall, these findings support the potential of functionalized nanocellulose coatings as emerging control strategies for high-touch surfaces in public and healthcare settings.



## P12.35 - SYNERGISTIC INHIBITION OF CANDIDA GLABRATA BIOFILMS BY ANTISENSE OLIGONUCLEOTIDES TARGETING PDR1 COMBINED WITH FLUCONAZOLE

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**Background & Aims:** The emerging threat of *Candida glabrata* infections (now renamed *Nakaseomyces glabratus*), exacerbated by the fungus's resistance to azole-based drugs, presents a global healthcare concern. *PDR1* plays a central role controlling the overexpression of efflux pumps in response to antifungal pressure, as well as membrane permeability, cell adhesion and stress adaptation. All these processes are critical for biofilm formation, which is central to resistance. To overcome this, antisense therapy offers a promising approach for targeting genes and modulating resistance at the mRNA level. Thus, the main objective is to evaluate whether an antisense oligonucleotide (ASO) targeting the *PDR1* transcription factor can effectively inhibit *Candida glabrata* growth and modulate fluconazole resistance in both planktonic and biofilm states.

**Methods:** A checkerboard assay was performed to assess the impact of *PDR1* downregulation on planktonic growth, while changes in mRNA expression levels were quantified by real-time quantitative PCR. The effects on biofilm formation were evaluated by measuring total biomass using crystal violet staining and determining cell viability through cultivable colony-forming units. In addition, the combined effect of the ASO and fluconazole (ASO-FLU) was analysed in both planktonic and biofilm cultures to determine potential synergistic interactions.

**Results:** *In vitro* planktonic assays demonstrated that treatment with the anti-*PDR1* ASO resulted in a statistically significant 20% reduction in *Candida glabrata* growth, accompanied by a 30% decrease in *PDR1* gene expression. Notably, the combined ASO-FLU treatment enhanced fluconazole activity, increasing its inhibitory effect by over 30% compared to fluconazole alone, effectively reducing by half the MIC<sub>50</sub> value. In biofilm assays, treatment with anti-*PDR1* ASO alone induced a measurable reduction in total biomass. However, when combined with fluconazole, biofilm reduction increased by around 30% compared to fluconazole alone after 24h and 48h. To note, while FLU showed minimal activity due to intrinsic resistance, and declining efficacy over time, the ASO-FLU combination consistently inhibited biofilm growth. A clear synergistic pattern was also observed when quantifying viable cells, confirming enhanced efficacy of the combination treatment.

**Conclusions:** These findings reveal a synergistic effect, in which ASO-mediated disruption of *PDR1*-dependent pathways enhances *Candida glabrata* susceptibility to azoles, both in planktonic cells and during biofilm development.



## P12.36 - DEVELOPMENT OF A NOVEL GRAFT COATING FOR THE PREVENTION OF VASCULAR GRAFT INFECTIONS

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**Background & Aims:** Vascular graft infections (VGIs) remain a severe postoperative complication associated with high mortality (20-80%) and substantial morbidity, including limb amputation, sepsis and pseudoaneurysm formation. Standard management usually requires graft explantation combined with targeted antimicrobial therapy, yet rising antibiotic resistance and the biofilm-forming capacity of many VGI-associated pathogens significantly limit therapeutic success. To address this unmet clinical need, we evaluated a novel antibiotic-loaded slow-release coating designed to enhance local antimicrobial availability. This study investigates whether this coating provides superior antibacterial activity compared with uncoated, conventionally antibiotic-soaked vascular grafts.

**Methods:** Gelatin-sealed polyester grafts were coated with a surface agnostic layer to which antibiotics were covalently attached at high density via unstable linkers enabling gradual drug release. Antimicrobial activity was assessed in vitro using an agar-plate model with prolonged incubation and repeated daily bacterial challenges to mimic early postoperative exposure.

**Results:** The coating was successfully applied and yielded a clear performance difference compared with antibiotic-soaked grafts. Soaked grafts showed an initial burst release followed by a rapid decline in activity, reaching sub-inhibitory antibiotic levels as early as day 3. These sub-inhibitory levels lasted for 10 more days, facilitating early bacterial attachment and favoring resistance development. These sub-inhibitory concentrations persisted for 10 more days, creating a prolonged window that may increase the risk of resistance development. In contrast, coated grafts exhibited markedly improved and sustained antimicrobial activity. They effectively inhibited bacterial growth and prevented early microbial adhesion, demonstrated by well-defined zones of inhibition. Remarkably, antimicrobial effects remained robust for at least 9 days, and coated grafts withstood multiple sequential bacterial challenges, highlighting their durability and prolonged functional efficacy.

**Conclusions:** This novel slow-release coating shows strong potential for early prevention of VGIs and represents a promising alternative to currently used antibiotic-soaked grafts. Upcoming in vivo studies will evaluate performance under physiological conditions. If successful, this technology could substantially reduce VGI incidence, improve clinical outcomes, and lower associated healthcare burdens.



## P12.37 - LOOKING INTO THE HUMAN MICROBIOME FOR NEW MULTIFUNCTIONAL PEPTIDES

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**Background & Aims:** The human microbiome represents a vast reservoir of unexplored bioactive peptides with potential therapeutic relevance. Antimicrobial peptides, antibiofilm peptides, and anti-inflammatory peptides are of particular interest due to their relevance in fighting prevalent biofilm-related infectious diseases. This study aimed to develop a computational pipeline to infer multifunctional peptide candidates derived from microbiome proteomic data. Focus was set on sequences with combined antimicrobial, antibiofilm, and anti-inflammatory potential.

**Methods:** Protein sequences were retrieved from the Human Microbiome Project (5–100 amino acids, canonical residues, deduplication). Functional inference was performed using three pre-trained deep learning models targeting antimicrobial, antibiofilm, and anti-inflammatory activities, respectively. Predictions were integrated through a conservative binary intersection and a percentile-based rank framework to define multifunctionality. BLASTp supported similarity evaluation against experimentally validated peptides in public databases (APD3, DBAASP, DRAMP). Final candidates were further characterized through physicochemical profiling and heuristic synthesis feasibility assessment.

**Results:** The computational strategy enabled the identification of six multifunctional peptide candidates supported by model agreement and probabilistic ranking with concurrent potential antibacterial, antibiofilm, and anti-inflammatory effects. Similarity analysis revealed only short partial alignments with validated peptides, suggesting potential uniqueness. Physicochemical profiling showed that selected sequences exhibited features consistent with bioactive peptides, including positive net charge, basic isoelectric points, and balanced hydrophobic residue fractions. Synthetic feasibility assessment indicated that most candidates fall within experimentally accessible ranges for peptide synthesis.

**Conclusions:** The proposed computational approach enabled the identification of potential multifunctional bioactive peptides from human microbiome data targeting important aspects of biofilm-related infections treatment. The selected candidates display physicochemical features consistent with bioactive host defence peptides and represent promising targets for further experimental validation.



## P12.38 - SCREENING OF NATURAL NATRIURETIC PEPTIDES DISPERSAL ACTIVITY ON PSEUDOMONAS AERUGINOSA BIOFILM IN A DYNAMIC FLOW-CELL MODEL

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**Background & Aims:** *Pseudomonas aeruginosa* is an opportunistic bacterial pathogen responsible for persistent and chronic infections. During these infections, the bacteria form aggregates/biofilms that enhance bacterial adaptability and tolerance to host immune system and antibiotics treatments. Thus, there is a need for alternative treatments targeting established biofilms. The use of exogenously added compounds to induce biofilm dispersion has emerged as a promising strategy since dispersed bacteria would then be more susceptible to antibiotics treatments. In previous studies we showed that the human atrial natriuretic peptide (hANP) and osteocrin induced marked dispersal of 24-h old *P. aeruginosa* biofilms, whereas the C-type and the brain natriuretic peptides have no dispersal effect. In the present study, we evaluated the potential dispersal activity of an array of natural natriuretic peptides (NP) on biofilm of *P. aeruginosa* established in a dynamic flow-cell system.

**Methods:** 24h-old *P. aeruginosa* biofilms were established in a dynamic flow-cell model and exposed 2h to oxidized and reduced forms of various natural NP at the concentration of 10 nM. Residual biomass was stained with Syto9 and analyzed using confocal laser scanning microscopy. Quantitative assessment of residual biomass was performed by images analysis using COMSTAT2 software. Statistical analyses were then performed to determine the significance of the dispersal activity of the tested peptides compared with the control condition.

**Results:** We showed that the rat atrial natriuretic peptide (rANP) triggered biofilm dispersal in the range of 57.7% with efficacy comparable to hANP. In contrast, we observed that several ANPs derived from other species, including crocodiles (~47.1%), snakes (~49.5%), and eels (~46.5%), exhibited slightly lower dispersal activity compared to hANP and rANP, while other ANPs were unable to induce biofilm dispersion. Moreover, we demonstrated that the oxidized form of these NPs seems to display greater antibiofilm efficacy than their reduced counterparts.

**Conclusions:** All these results show that NP could be very interesting adjuvants for the development of new anti-biofilm agents. The variability in dispersal activity observed within this peptide family suggests a structure-activity relationship that warrants further investigation. The results obtained through this screening will be used to optimize the sequence of new synthetic NP for their biofilm dispersal effect.



## P12.39 - CRACKING PSEUDOMONAS AERUGINOSA BIOFILMS: SMALL-MOLECULE LIBRARY STRIKES BACK

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**Background & Aims:** *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic pathogen notorious for causing persistent infections due to strong biofilm forming capability. It is frequently associated with hospital-acquired infections and poses significant threat to immunocompromised patients. Biofilms are structured bacterial communities embedded in a protective extracellular matrix that provides resistance to antibiotics and immune clearance. Chronic biofilm-related infections represent a major clinical challenge, as cells in biofilms can be up to 1000 times more resistant to antibiotics than planktonic cells. Chronic biofilm infections challenge treatment, driving the need for antibiofilm compounds to disrupt formation and enhance efficacy. In this study, 100 clinical *P. aeruginosa* isolates underwent whole-genome sequencing (WGS) and phenotypic screening for biofilm formation. Moreover, we screened the University of Ljubljana Faculty of Pharmacy compound library for biofilm formation inhibitors using the reference strain PAO1.

**Methods:** Biofilm formation of various clinical isolates of *P. aeruginosa* strains was quantified via crystal violet staining assay. The biofilm formation index categorized isolates as strong, moderate, weak, or non-biofilm formers. WGS was performed for all isolates. The compound library was also screened for antibiofilm activity against the reference strain PAO1.

**Results:** In this study, 100 *P. aeruginosa* isolates originating from various clinical samples and animal species (mainly pets, predominantly dogs and cats, but also a snake and a sea lion) underwent phenotypic screening for biofilm formation. The isolates exhibited high genetic diversity according to WGS. Phenotypically, 53% were strong biofilm formers, 12% moderate, 13% weak, and 22% non-formers. Several compounds significantly inhibited PAO1 biofilm formation (up to 93,7 % inhibition for top hits), identifying promising candidates for further development into potent antibiofilm compounds.

**Conclusions:** These findings highlight the substantial genetic diversity of *P. aeruginosa* isolates from animals and emphasize the high prevalence of strong biofilm-forming phenotypes, which may contribute to persistence and complicate treatment in veterinary infections. The identification of small molecules with antibiofilm activity against PAO1 highlights the value of phenotypic screening approaches for discovering novel antibiofilm agents.



## P12.40 - MONOBACTAMS AS DOUBLE AGENTS: KILLING BACTERIA AND DISARMING BIOFILMS

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**Background & Aims:** Antimicrobial resistance is a major global health concern, further exacerbated by the ability of bacteria to form biofilms, which confer increased tolerance to antibiotic treatment. Monobactams are  $\beta$ -lactam antibiotics with intrinsic stability against metallo- $\beta$ -lactamases, making them attractive scaffolds for further development. This study aimed to design and evaluate monobactam-based conjugates that combine antibacterial activity with biofilm-targeting properties by interfering with quorum sensing or mimicking nitric oxide-mediated signaling.

**Methods:** Two series of aztreonam-based conjugates were synthesized. The first series comprised nitroxide derivatives designed to mimic nitric oxide, while the second included acylhomoserine lactone (AHL) and acylhomocysteine lactone analogues targeting quorum sensing pathways. The conjugates were prepared via amide bond formation and, for selected AHL derivatives, additionally as sulfonic acid esters. Antibacterial activity was evaluated by determining minimum inhibitory concentrations (MICs) against selected ESKAPE pathogens. Biofilm inhibition and disruption were assessed in *Escherichia coli*, *Pseudomonas aeruginosa* PAO1, and *Acinetobacter baumannii*, using both reference strains and clinical isolates. Inhibition of penicillin-binding protein 1b (PBP1b) from *Streptococcus pneumoniae* was determined, and crystal structures of selected conjugates in complex with PBP1b were resolved.

**Results:** Several conjugates showed antibacterial activity comparable to aztreonam. In contrast, enzyme inhibition studies demonstrated that the majority of conjugates inhibited PBP1b more potently than aztreonam. Structural analysis revealed additional interactions within the active site arising from the conjugated moieties, supporting the observed increase in target affinity. Multiple compounds demonstrated inhibition of biofilm formation across the tested strains, whereas activity against established biofilms was less pronounced but still indicated partial disruption. Notably, activity profiles varied between strains, with no single conjugate consistently demonstrating superior efficacy across all species.

**Conclusions:** Monobactam conjugation with biofilm-targeting moieties expands the functional profile of  $\beta$ -lactam antibiotics by combining enzyme inhibition with interference in biofilm formation. Despite slightly reduced antibacterial activity, enhanced inhibition of the model enzyme PBP1b, together with structural evidence of additional binding interactions, indicates improved target engagement. The observed discrepancy between enzyme inhibition and antibacterial activity is therefore likely driven by limited permeability and/or active efflux. These findings support further optimization of monobactam conjugates to balance physicochemical properties with antibacterial and antibiofilm activity.



## **P12.41 - ANTIMICROBIAL FUNCTIONALIZATION OF AN INJECTABLE CALCIUM PHOSPHATE CEMENT FOR PREVENTION OF ORTHOPAEDIC IMPLANT- ASSOCIATED BIOFILM INFECTIONS**

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**Background & Aims:** Orthopaedic implant-associated infections remain a major complication in bone reconstruction procedures, largely driven by bacterial biofilm formation on implanted materials. Calcium phosphate cements (CPCs) are promising bone substitutes due to their osteoconductivity, biodegradability, and injectability. However, they lack intrinsic antimicrobial activity. This work aims to functionalize an injectable CPC, Neocement® Inject P from Bioceramed S.A., to provide antibacterial properties while preserving its biological and mechanical performance. The study evaluates the incorporation of antibiotics (gentamicin, vancomycin, and rifampicin) and the ionic liquid (IL) 1-ethyl-3-methylimidazolium hydrogen sulfate as antimicrobial agents to develop a multifunctional cement capable of preventing biofilm-associated infections.

**Methods:** Modified cement formulations were prepared by incorporating antimicrobial agents into the Neocement® Inject P powder prior to mixing. Cylindrical specimens were produced for characterization assays. Cytocompatibility was assessed through an indirect MTS assay, to evaluate the effect of the modified cements on the osteoblast metabolic activity. Antimicrobial activity was evaluated via colony forming units (CFU) counting using *Staphylococcus aureus* and *Escherichia coli* as models. Physicochemical characterization of the samples was performed using Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) to evaluate chemical composition and phase formation, while scanning electron microscopy (SEM) was used to analyze surface morphology and microstructure. Additional characterization assays are still ongoing, which includes drug release studies in PBS, interporosity analysis, mechanical properties, and setting time.

**Results:** FTIR analysis showed that the characteristic functional groups of the calcium phosphate cement matrix remained unchanged after the incorporation of antimicrobial agents. Antibiotic-loaded formulations demonstrated antibacterial activity against both strains. Cytotoxicity results indicated that IL and antibiotics were non-toxic at the concentration ranges studied.

**Conclusions:** These results suggest that functionalization of Neocement® Inject P with antibiotic agents provide antibacterial activity while maintaining cytocompatibility at appropriate concentrations. This approach shows potential for the development of injectable biomaterials capable of preventing biofilm-associated orthopaedic infections.



## P12.42 - PROPOLIS-LOADED CHITOSAN NANOPARTICLES AS A SUSTAINABLE STRATEGY FOR THE TREATMENT OF MIXED PARACOCCIDIOIDES BRASILIENSIS-PSEUDOMONAS AERUGINOSA BIOFILMS

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**Background & Aims:** Patients with paracoccidioidomycosis often require prolonged hospitalization, increasing the risk of secondary infections by opportunistic pathogens such as *Pseudomonas aeruginosa* (Pa). In this context, pre-formed biofilms of *Paracoccidioides brasiliensis* (Pb) may facilitate subsequent bacterial colonization, leading to mixed-species biofilms with enhanced persistence and antimicrobial tolerance. Given the limited efficacy of conventional therapies against polymicrobial biofilms, this study aims to investigate the biofilm formation of Pb and Pa and to evaluate the antimicrobial potential of chitosan nanoparticles loaded with brown Propolis (PMA), specifically against pre-formed fungal-bacterial biofilm.

**Methods:** Chitosan nanoparticles loaded with propolis (NP-PMA) were developed and characterized using dynamic light scattering. Monospecies and mixed biofilms were formed under simultaneous and pre-formed conditions, based on the method described by Medina-Alarcón et al. (2021), using an inoculum of 10<sup>6</sup> CFU/mL in Brain Heart Infusion (BHI) medium. Antimicrobial treatments with PMA and NP-PMA were applied to pre-formed mixed Pb-Pa biofilm and incubated for 48 h. Biofilm cell viability, total biomass, and extracellular matrix production were evaluated using resazurin, crystal violet, and safranin staining, respectively. Structural analysis was performed by scanning electron microscopy (SEM).

**Results:** In mono-species biofilms, Pa exhibited higher cellular viability and biomass during early stages, whereas Pb showed a slower but sustained increase over time. Simultaneous biofilm formation resulted in enhanced cellular viability compared to monospecies biofilms. Pre-formed mixed biofilms exhibited the highest biomass accumulation, along with increased extracellular matrix production, possibly associated with the production of extracellular polymeric substances, which may provide additional protection to Pb within the mixed biofilm. SEM analysis corroborated these findings, revealing denser and more complex biofilm structures in mixed-species conditions. Treatment assays demonstrated that chitosan nanoparticles loaded with propolis (NP-PMA) promoted inhibition levels comparable to or higher than free propolis, particularly in reducing cellular viability and extracellular matrix content in pre-formed mixed biofilms.

**Conclusions:** Both microorganisms form structured and metabolically active biofilms, and these interkingdom interactions may contribute to increased persistence and reduced susceptibility to antimicrobial therapies. Nanoparticle-based treatment showed promising activity against pre-formed mixed biofilms, supporting its potential as an alternative strategy for controlling fungal-bacterial biofilm-associated infections.



## P12.43 - EFFECT OF CITRIC ACID-BASED COMPOUNDS WITH SILVER IONS AGAINST BIOFILMS FORMED BY LISTERIA MONOCYTOGENES AND PSEUDOMONAS FLUORESCENS ISOLATED FROM THE FOOD INDUSTRY

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**Background & Aims:** *Listeria monocytogenes* and *Pseudomonas fluorescens* are commonly isolated in the food industry. While the former represents a food safety concern, the latter is responsible for food spoilage. The biofilm-forming abilities of these microorganisms facilitate their persistence in food processing environments. Along with traditional antibiofilm strategies, innovative compounds based on citric acid and silver ions can be employed. In this study, we assessed the effect of two citric acid-silver ion formulations against biofilms formed by *L. monocytogenes* and *P. fluorescens* isolated from food industries.

**Methods:** For each species, three biofilm-forming strains were selected, including one ATCC and two wild-type strains obtained from dairy (*P. fluorescens*) and meat (*L. monocytogenes*) environments. Two citric acid-silver ion formulations, SC30 and SC2400, were tested. The Minimum Inhibitory Concentration (MIC) of the compounds was determined following the Clinical Laboratory Standard Institute (CLSI) protocol. Based on MIC values, the Minimum Biofilm Eradication Concentration (MBEC) was assessed with the 96-well MBEC device. Finally, the effect of the formulations, at the optimal MBEC values, was tested using the macro- method assay on 24-hour biofilms formed on stainless steel and polycarbonate.

**Results:** The MIC values for *L. monocytogenes* were higher than those observed for *P. fluorescens*. The MBEC assay showed that *L. monocytogenes* biofilms were resistant to SC30, as wild-type *Pseudomonas*, and sensitive to high concentrations of SC2400 (MBEC between 100% and 75%). The ATCC strain of *P. fluorescens* was sensitive to high concentrations of SC30 (MBEC at 50%) and low concentrations of SC2400 (MBEC at 3.12%). The results were confirmed by the macro-method, since the maximum reduction in viable detached cells was observed in all cases. For *L. monocytogenes*, the reduction was  $\geq 7.06$  Log CFU/cm<sup>2</sup> on polycarbonate and  $\geq 6.69$  Log CFU/cm<sup>2</sup> on stainless steel. For *P. fluorescens* ATCC, the reduction was  $\geq 8.18$  Log CFU/cm<sup>2</sup> on polycarbonate and  $\geq 7.75$  Log CFU/cm<sup>2</sup> on stainless steel.

**Conclusions:** Citric acid-silver ion compounds showed promising antibiofilm properties, although their efficacy was influenced by both the bacterial species and the formulation. Further trials should be performed to assess whether a comparable effect on viable biofilm cells can be achieved at lower concentrations.



## P12.44 - BIOACTIVITY-GUIDED FRACTIONATION OF THE ANDEAN PLANT MACLEANIA RUPESTRIS ENHANCES ITS ANTI-BIOFILM ACTIVITY AGAINST STAPHYLOCOCCUS AUREUS

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**Background & Aims:** Biofilms are a major contributor to the persistence and clinical severity of microbial infections due to their heightened tolerance to antimicrobial agents. Particularly, *Staphylococcus aureus* biofilms are among the leading causes of skin and soft tissue infections, bacteremia and chronic infections associated with medical devices. In this context, natural products derived from medicinal plants represent an underexplored source of anti-biofilm agents. *Macleania rupestris* (Ericaceae) is an Andean fruit-bearing species traditionally used by rural communities in South America for its medical properties. This study aimed to evaluate the anti-biofilm activity of *M. rupestris* ethanolic extract and identify bioactive fractions responsible for these effects.

**Methods:** The biofilm inhibition activity of our *M. rupestris* extract was evaluated using the standard crystal violet (CV) staining method. *S. aureus* was grown with increasing extract concentrations (0.1 - 30 mg/mL) during 24 hours in 96-well plates. Simultaneously, the biocompatibility of the extract was assessed through erythrocyte hemolysis assays and RAW 264.7 macrophage inflammation models. The crude extract was fractionated using Bond Elut solid phase extraction (SPE) and each fraction was individually screened for anti-biofilm activity. Finally, all active fractions were chemically characterized by HPLC-DAD-MS/MS analysis.

**Results:** The *M. rupestris* extract displayed important biofilm inhibition activity against *S. aureus* ( $71 \pm 3.31\%$  biofilm inhibition). The hemolytic activity was negligible ( $<5\%$  at all tested concentrations) and the extract displayed moderate anti-inflammatory activity ( $> 50\%$  reduction in Nitric oxide production), indicating high biocompatibility. A total of 24 fractions were obtained after SPE-derived fractionation. Fractions 8 and 9 exhibited almost full inhibition activity against *S. aureus* biofilm ( $99 \pm 0.65\%$  and  $98 \pm 0.19\%$ , respectively) indicating that fractionation markedly enhanced the biofilm-inhibition activity of the extract. Chemical profiling revealed flavonoids and phenolic acids as dominant constituents of the fractions that exhibited enhanced anti-biofilm effects.

**Conclusion:** Bioactivity-guided fractionation of *M. rupestris* significantly enhanced its biofilm inhibition properties against *S. aureus* and enabled the identification of plant-derived metabolites of interest. These findings highlight the potential of Andean plant-derived metabolites as promising candidates for the development of alternative antibiofilm agents.



## P12.45 - ANTIFUNGAL ACTIVITY OF A NEW CLASS OF 2-AMINOIMIDAZOLE AZO DYES ON *C. KRUSEI* AND *C. AURIS* BIOFILMS

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**Background & Aims:** Invasive infections caused by *Candida* spp. pose a significant burden in healthcare settings, with non-*albicans* species increasingly contributing to disease. *C. krusei* (intrinsically resistant to fluconazole) and *C. auris* (often multidrug-resistant) are among the WHO priority pathogens and exemplify biofilm-forming yeasts that persist in healthcare settings. The imidazole moiety has been linked to increased antimicrobial activity in azo dyes. As such, this new class of 2-aminoimidazole azo dyes arises as a potential control strategy for fungal infections, due to its established antimicrobial activity against pathogenic yeasts, namely *C. neoformans* and *C. krusei*.

**Methods:** This class of 2-aminoimidazole azo dyes was obtained through the reaction of imidazole-based amidrazone precursors with secondary amines, with optimizations of the reported method. The antifungal activity of this compound was evaluated against *C. krusei* and *C. auris* strains, determining its Minimum Inhibitory Concentration (MIC), according to the European Committee on Antimicrobial Susceptibility Testing protocols for yeasts. Then, the effect of the compound on *Candida* spp. biofilm formation was evaluated after 24 h, with the activity quantified by colony-forming units (CFUs) enumeration.

**Results:** Compound A inhibited the growth of the tested strains, with different levels of activity. MIC values ranged from 8 to 16 µg/mL, with *C. auris* appearing slightly more susceptible than *C. krusei*. Given the increased tolerance of biofilm-associated cells, higher concentrations of compound (2-4x MIC) were used in biofilm assays. For *C. krusei*, exposure to the highest concentration of compound (32 µg/mL) for 24 h resulted in a significant inhibitory effect on biofilm formation (2.0 log reduction,  $P < 0.0001$ ), with no significant effect observed with 16 µg/mL. In contrast, *C. auris* was markedly more susceptible, showing a significant inhibition of biofilm formation in the presence of the compound, with reductions of approximately 2.6 and 4.5 log with 16 µg/mL and 32 µg/mL of compound, respectively ( $P < 0.0001$ ).

**Conclusions:** Our results showed great effectiveness of compound A in preventing biofilm formation. Considering this, the design of various 2-aminoimidazole azo dyes with potentially improved activity against *Candida* species can be addressed.



## P12.46 - POLYMERIC NANOFORMULATIONS ENCAPSULATING BIOACTIVE AGENTS EMBEDDED IN A HYDROGEL FOR MULTIFUNCTIONAL CHRONIC WOUND HEALING

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**Background & Aims:** Chronic wounds represent a severe global healthcare challenge, characterized by prolonged inflammation driven by microbial colonization, impaired angiogenesis, and excessive oxidative stress. Conventional treatments often fail against this multifactorial pathology, highlighting the urgent need for advanced solutions. Integrating polymeric nanoformulations within hydrogels offers a promising approach. Hydrogels provide a moisture-retaining biomimetic scaffold, while nanoformulations ensure protective encapsulation and sustained therapeutic release. This work aims to develop a multifunctional dressing by embedding Poly(L- lactic acid) (PLLA) nanoformulations, loaded with the ionic liquid (IL) 1-ethyl-3-methylimidazolium hydrogen sulfate ([EMIM][HSO<sub>4</sub>]), a compound recognized for its high physicochemical stability and antimicrobial properties, into a hydrogel network, targeting structural stability and antimicrobial performance to actively promote wound healing.

**Methods:** [EMIM][HSO<sub>4</sub>]-loaded PLLA nanoformulations (containing 5% and 10% (w/v) IL) were synthesized using a double emulsion solvent evaporation method. To evaluate their clinical potential, these nanocarriers are incorporated into alginate/agarose and chitosan/alginate hydrogel matrices. Physicochemical and morphological properties are characterized by Dynamic Light Scattering (DLS), Fourier-Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). Functional and biological evaluations include *in vitro* IL release studies, cytotoxicity assays, and Colony Forming Unit (CFU) counts against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Staphylococcus epidermidis*.

**Results:** DLS analysis confirms 4-week physical stability of PLLA nanoformulations, with optimal sizes ranging 197-260 nm and monodisperse populations (Polydispersity Index, PDI < 0.2). FTIR and absorption spectrometry analysis confirmed IL encapsulation, and SEM revealed spherical morphology and homogeneous distribution within the hydrogel matrix. Regarding antimicrobial activity, empty PLLA nanocarriers show intrinsic efficacy, achieving up to 2.8 and 3.6 log reductions against *S. aureus* and *P. aeruginosa*, respectively, whereas 10% IL-loaded formulations achieve 5.2 and 6.7 log reductions against *E. coli* and *P. aeruginosa*. Once embedded, the composites demonstrate sustained IL release, alongside high cytocompatibility with cell viability above 70%.

**Conclusions:** [EMIM][HSO<sub>4</sub>]-loaded PLLA nanoformulations embedded within polysaccharide-based hydrogels represent a promising multifunctional strategy for chronic wound management. This synergistic platform is expected to overcome persistent bacterial colonization through sustained antimicrobial action, while the moisture-retaining biomimetic scaffold provides an optimal microenvironment to support tissue regeneration. This work has been funded by the FCT-funded project PhysiPlant (COMPETE2030-FEDER-00896400).



## P12.47 - CLEC3A-DERIVED ANTIMICROBIAL PEPTIDES AS NOVEL AGENTS AGAINST FUNGAL BIOFILMS: MECHANISTIC INSIGHTS AND CLINICAL IMPLICATIONS

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**Background & Aims:** Antifungal resistance, poses a major threat to global health, with *Candida auris*, *Candida albicans*, and *Cryptococcus neoformans* recently classified by the WHO in the fungal priority pathogens list as critical priority pathogens. Over the last years, fungal biofilm and especially *Candida* biofilm infections have become a growing problem due to the frequent use of immunosuppressive drugs, the increased implantation of various medical devices, and an aging population. Antimicrobial peptides (AMPs) have emerged as promising candidates against these pathogens due to their broad-spectrum activity and membrane disruptive mechanisms.

**Methods:** In this study, the antifungal efficacy of two CLEC3A-derived antimicrobial peptides, HT-47 and WRK-30, was evaluated in comparison to the well-known AMP LL-37 and the antifungal drugs amphotericin B and rezafungin using viable count assays as well as scanning and transmission electron microscopy (SEM & TEM). Furthermore, their anti-biofilm efficacy was investigated using crystal violet and Alamar Blue viability assays.

**Results:** HT-47 and WRK-30 exhibited potent antifungal effects against *C. albicans*, *C. auris*, and *C. neoformans* with MIC50 values comparable to or lower than amphotericin B. SEM and TEM revealed extensive membrane and subcellular damage in peptide-treated fungal cells. Both peptides lead to a more potent inhibition of *C. auris* and *C. albicans* biofilm formation, compared to amphotericin B, shown by the reduction in biomass of treated biofilms. HT-47 and WRK-30 also significantly reduced cell viability in preformed *C. auris* biofilms.

**Conclusions:** CLEC3A-derived AMPs HT-47 and WRK-30 exhibit strong antifungal properties against both planktonic and biofilm-forming cells, highlighting their potential as therapeutic candidates for combating multidrug-resistant pathogens, including *C. auris*.



## P12.48 - CROSS-KINGDOM COMMUNICATION: EFFECTS OF GIARDIA LAMBLIA EXTRACELLULAR VESICLES ON BACTERIAL BIOFILMS

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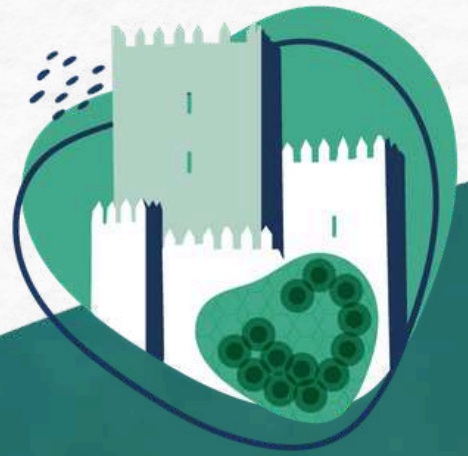
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**Background & Aims:** *Giardia lamblia* trophozoites release extracellular vesicles (EVs) containing diverse molecular cargo, including proteins, nucleic acids, and virulence-associated factors that modulate host cell signaling and contribute to immune regulation, parasite survival, and pathogenesis. Although parasite-derived EVs are increasingly recognized as mediators of host-parasite interactions, their effects on intestinal bacterial biofilms remain poorly understood. Biofilms are critical for bacterial persistence, antimicrobial resistance, and intestinal homeostasis. This study aimed to investigate the effects of *G. lamblia* EVs on biofilm formation by representative commensal and pathogenic intestinal bacteria.

**Methods:** *G. lamblia* trophozoites [strain WB, clone 6 (ATCC 30957)] were cultured at 37 °C in Keister's modified TYI- S-33 medium. EVs were isolated from cultures using the exoEasy™ Maxi Kit and characterized by Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscopy (TEM), and Electrophoretic Light Scattering (ELS). Bacterial strains including *Bacillus subtilis* (NRS 231), *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (C122), *Enterococcus faecalis* (ATCC 29212), and *Salmonella* spp. were grown in Lysogeny Broth (LB) broth. Overnight cultures were adjusted to 0.5 McFarland and inoculated into 96-well flat-bottom polystyrene microtiter plates to allow single-species biofilm formation. Biofilm biomass was quantified by crystal violet staining and viable counts were determined by serial dilution and plating. The effects of intact EVs and EV extracts on preformed biofilms were evaluated after 24 h of incubation at 37 °C using crystal violet and viable bacterial counts.

**Results:** The crystal violet assay confirmed biofilm formation by all bacterial species, with absorbance values ranging from 1.59 to 2.47. Viable bacterial counts ranged from  $1.0 \times 10^7$  to  $8.32 \times 10^7$  CFU/mL. Based on absorbance criteria ( $OD_a > 4 \times OD_c$ ) and viable biomass ( $>7 \log$  CFU/mL), all tested strains can be classified as strong biofilm producers. *Giardia* EVs were successfully isolated and displayed a predominant population of exosome-like vesicles. Evaluation of the effects of *Giardia* EVs on established biofilm is ongoing.

**Conclusions:** Bacterial biofilms were successfully established and quantified, providing a robust platform to evaluate the impact of *Giardia* EVs. These findings support further exploration into parasite-bacteria interactions and the potential application of EVs in strategies targeting bacterial biofilms and infection control.



# 12

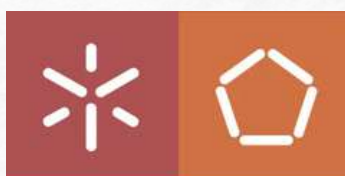
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