Antibiotics
2026
Conference

Antibiotics 2026: Advances in Antimicrobial Action and Resistance

11–14 May 2026 | Barcelona, Spain

Program and Abstract Book

Contents

Welcome from the Chairs

Dear Colleagues,

Welcome to **Antibiotics 2026—Advances in Antimicrobial Action and Resistance**, taking place in Barcelona, Spain, from the 11th to the 14th of May 2026

This conference brings together researchers, industry professionals, and experts from across the globe to share the latest advancements, exchange ideas, and foster collaboration in the field of antimicrobials, antimicrobial resistance, and innovative therapeutic strategies.

Over four days, the programme will cover a broad range of topics, including antimicrobial resistance mechanisms, antimicrobials and One Health, ethnopharmacology in research and treatment, traditional and emerging approaches in the discovery of new antimicrobial agents, and advances in antimicrobial stewardship and clinical practice. The scientific programme features keynote lectures, oral presentations, and poster sessions, providing a dynamic forum for discussing cutting-edge research, current challenges, and future directions.

We encourage you to engage fully - present your work, contribute to discussions, and connect with colleagues from around the world. At the same time, don't forget to enjoy Barcelona during the free afternoon on Wednesday, with its rich culture, history and cuisine!

We wish you a stimulating and productive conference and are delighted to welcome you to Antibiotics 2026.

Sincerely,

Prof. Dr. Manuel Simões
Prof. Dr. Jordi Vila
Dr. Marc Maresca

Event Chairs

Prof. Dr. Manuel Simões
Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal

Prof. Dr. Jordi Vila
Department of Clinical Microbiology, Hospital Clinic, Spain

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Dr. Marc Maresca
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Keynote Speakers

Dr. Mariana Castanheira
Element Materials Technology, JMI Laboratories, USA

Prof. Timothy R. Walsh DSc OBE
Ineos Oxford Institute for Antimicrobial Research, University of Oxford, UK

Invited Speakers

Dr. Jean-Michel Bolla
INSERM, Aix-Marseille University, France

Prof. Dr. Efstathios Giaouris
University of the Aegean, Greece

Prof. Dr. Walter Luyten
Department of Biology, KU Leuven, Belgium

Prof. Dr. Luísa Peixe
UCIBIO, University of Porto, Portugal

Prof. Dr. Patrice Nordmann
Department of Medicine, University of Fribourg, Switzerland

Prof. Dr. Adnane Remmal
Laboratory for the Valorization of Aromatic Plants, Sidi Mohamed Ben Abdellah University, Morocco

Prof. Dr. Aurélie Tasiemski
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Prof. Dr. Carmen Torres Manrique
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Prof. Dr. Evelina Tacconelli
Infectious Diseases Center for Translational Research (ID-CARE), University of Verona, Italy

Dr. Markus Weingarth
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Center for Biomedical Research of La Rioja (CIBIR), Spain

Prof. Domenico Schillaci
Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

Prof. Dr. Sara M. Soto González
Institute for Global Health (ISGlobal), Hospital Clínic, Universitat de Barcelona, Spain

Antibiotics 2026: Advances in Antimicrobial Action and Resistance

Monday 11 May 2026

CEST	Session
From 12:45	**Check-in**
13:45-14:00	Opening Ceremony
14:00-16:00	**Innovation in Antimicrobial Stewardship and Optimized Clinical Strategies** Invited Speaker - Jean-Michel Bolla Selected Presentations Invited Speaker - Evelina Tacconelli
16:00-16:30	Coffee Break
16:30-17:45	**Antimicrobials, Antimicrobial Resistance, Ethnopharmacology and One Health (part 1)** Selected Presentations Invited Speaker - Aurélie Tasiemski

Program

Antibiotics 2026: Advances in Antimicrobial Action and Resistance

Tuesday 12 May 2026

CEST	Session
08:50-09:45	**Keynote Speaker - Mariana Castanheira**
09:45-10:45	**Antimicrobial Resistance Mechanisms** Selected Presentations
10:45-11:15	Coffee Break
11:15-13:15	**Conventional and Novel Approaches in the Discovery of New Antimicrobial Agents (part 1)** Invited Speaker - Patrice Nordmann Selected Presentations Invited Speaker - Markus Weingarth
13:15-15:00	Group Photo Lunch Break & Poster Session A
15:00-16:00	**Conventional and Novel Approaches in the Discovery of New Antimicrobial Agents (part 2)** Selected Presentations
16:00-16:30	Coffee Break & Poster Session A
16:30-18:15	**Antimicrobials, Antimicrobial Resistance, Ethnopharmacology and One Health (part 2)** Invited Speaker - Efstathios Giaouris Selected Presentations

Antibiotics 2026: Advances in Antimicrobial Action and Resistance

Wednesday 13 May 2026

CEST	Session
08:50-09:45	**Keynote Speaker - Timothy Walsh**
09:45-10:45	**Conventional and Novel Approaches in the Discovery of New Antimicrobial Agents (part 3)** Selected Presentations
10:45-11:15	Coffee Break & Poster Session B
11:15-13:15	**Antimicrobials, Antimicrobial Resistance, Ethnopharmacology and One Health (part 3)** Invited Speaker - Luisa Peixe Selected Presentations Invited Speaker - Carmen Torres
13:15-15:00	Lunch Break & Poster Session B
15:00-20:00	**Afternoon At Leisure** →
at 20:00	Conference Dinner →

Thursday 14 May 2026

CEST	Session
09:30-11:30	**Antimicrobials, Antimicrobial Resistance, Ethnopharmacology and One Health (Part 4)** Invited Speaker - Adnane Remmal Selected Presentations Invited Speaker - Walter Luyten
11:30-12:00	Coffee Break
12:00-12:30	**Closing & Award Ceremony**

General Information

Antibiotics 2026—Advances in Antimicrobial Action and Resistance will be held at the UPF - Barcelona School of Management, in Barcelona 11-14 May 2026. Over the course of four days, we will explore a wide range of topics, including antimicrobial resistance mechanisms, antimicrobials and One Health, ethnopharmacology in antimicrobial research and treatment, conventional and novel approaches in the discovery of new antimicrobial agents, and innovation in antimicrobial stewardship and optimized clinical strategies, through keynote lectures, oral presentations, and poster sessions.

Conference Venue

UPF - Barcelona School of Management (BSM)
C/ de Balmes, 132, 134, 08008 Barcelona, Spain

Registration Desk

The desk for registration, information and distribution of documents will open from 12:45h on 11 May 2026.

Certificate of Attendance

Participants of the event will be able to download an electronic Certificate of Attendance by accessing their dashboards on Sciforum.net once the event is concluded. The certificates will be found under the “My Certificates" category.

Disclaimer

Delegates will receive a name-badge at the Information Desk, upon registration. The badge must be worn prominently in order to gain access to the congress area during the event. Admission will be refused to anyone not in possession of an appropriate badge.

Insurance

The organizers do not accept liability for personal accidents, loss, or damage to private property incurred because of participation in Antibiotics 2026—Advances in Antimicrobial Action and Resistance. Delegates are advised to arrange appropriate insurance to cover travel, cancellation costs, medical, and theft or damage of belongings.

The Venue

The Barcelona School of Management (BSM) is situated in a bustling area of the city, near the heart of Barcelona, with easy access to both urban and suburban regions. Located on C/ de Balmes, BSM is in close proximity to several of the city's most iconic landmarks. Just a short walk away, you can find the renowned architectural masterpieces La Pedrera and Casa Batlló, both designed by the famous architect Antoni Gaudí. Additionally, the prestigious Passeig de Gràcia, known for its high-end shops and vibrant atmosphere, is nearby, making BSM an ideal location for experiencing the rich culture and dynamic energy of Barcelona.

Conference Dinner

We are excited to announce that the Conference Dinner Reception will be held at the prestigious **Catalonia Boulevard**, located in the heart of Barcelona. Enjoy a delightful evening with contemporary Catalan cuisine in a vibrant setting next to iconic landmarks such as **La Pedrera**, **Passeig de Gràcia**, **Casa Batlló**, and **Plaça Catalunya**.

- **Time:** Wednesday, 13 May 2026, at 20:00 CET
- **Location:** Catalonia Boulevard. C/ del Rosselló, 249, 08008 Barcelona
- **Main course options:** meat, fish or vegetarian (view the full menu here)

Join us for an unforgettable dining experience in one of Barcelona's most renowned locations!

More information here.

Conference Secretariat

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Emergency Information

All emergencies in Barcelona: 112

Fire brigade: 080

Accident / Ambulance: 061

National Police: 091

Local Police: 092

Abstracts — Talks

Keynote and Invited Talks

Keynote Speaker

Where Resistance Meets Drug Development: Lessons from 20 Years of Antimicrobial Research

Mariana Castanheira

[1] *Element Materials Technology, JMI Laboratories, USA*

Over the past 20 years, the study of antimicrobial resistance mechanisms has evolved from a largely descriptive discipline into a predictive science. The SENTRY Antimicrobial Surveillance Program—which collects and characterizes approximately 30,000 clinical isolates annually from around the world—has helped define the modern understanding of resistance epidemiology and directly supported the development and positioning of many antibacterial agents approved in the last 15 years.

A major inflection occurred around 2010, when the development of β-lactam/β-lactamase inhibitor combinations began to require precise differentiation of activity against specific resistance mechanisms. This shift transformed the field, driving a move from retrospective observation to systematic, prospective surveillance integrated with advanced molecular characterization.

In this presentation I will discuss how the genetic characterization studies performed using the SENTRY Program platform aided the differentiation of antimicrobial agents and provided support for clinical decisions. Ultimately, these insights underscore a fundamental shift in antimicrobial therapy—from choosing drugs based on organisms to selecting them based on resistance mechanisms.

Keynote Speaker

Clinical and Financial Burden of Global AMR

Timothy Walsh

[1] *Department of Biology, University of Oxford, UK*
[2] *Ineos Oxford Institute for Antimicrobial Research (IOI), UK*

The UNGA on AMR in September 2024 provided a seminal moment to recapture the momentum generated in 2015 but sadly, due to a number of reasons it did not resonate. The diminished funding to the UN has curbed the ambition of most WHO/FAO AMR programs, and the discontinued funding of the Fleming Fund by the UK GOV signals a shift in international and international priorities. Whilst bacteria are increasingly capable of communicating with each other, the human race seems to lack such unity to produce tractable solutions - the AMR solutions are known and tractable but as a global medico-scientific community we lack vision and belief to come together to seriously address AMR. The amount spent on AMR is approx. X1000 less than that spent on arms/defence, yet the number of deaths due to AMR is 100-fold to that of international conflict – and thus, our priorities are not aligned with the risk. Ironically, and tragically, international conflict further worsens the local issues of AMR infection. Climate change and environmental pollution with plastics also exacerbate the AMR problem. The antibiotic pipeline is challenged by relentlessly increasing levels of AMR which cuts across all UN SDGs and yet, still remains largely invisible to the general public. The successful arrest of global AMR is not a knowledge-gap or logistical issue, but a financial issue – we know what the solutions are! My lecture will attempt to bring all these factors into play but also discuss possible solutions and the pivotal role that outside players will play in leading on the world-stage to tackle global AMR.

Invited Speaker

Efflux Pump-Based Diagnostic Approach for Accelerated Antibiotic Resistance Detection

Jean-Michel Bolla

[1] *INSERM and UMR-MD1, Faculty of Pharmacy, Aix-Marseille University, France*

Antibiotic resistance is a major global issue requiring coordinated action from researchers, industry, and healthcare systems. Affecting human, animal, and environmental health, it is now addressed through the “One Health” approach. The World Health Organization has identified priority pathogens capable of developing multidrug resistance and emphasizes the urgent need for new treatments, regulatory strategies, and diagnostic tools.

Rapid and accessible detection methods are essential, particularly for low-resource settings. Currently, the antibiogram remains the standard method but requires 48–72 hours, limiting timely decision-making. Faster alternatives are therefore needed.

Among resistance mechanisms, efflux pumps—identified since the 1980s—play a key role. These systems expel toxic compounds, including antibiotics and antiseptics, preventing them from reaching effective intracellular concentrations. Their overproduction is frequently observed in multidrug-resistant bacteria, making them an important target for detection. Identifying strains with hyperactive efflux can improve therapeutic choices and help limit the spread of resistance.

Based on the ability of efflux pumps to transport diverse molecules, we investigated fluorescent phenazinium derivatives as potential substrates. Compounds synthesized by Olivier Siri’s team were evaluated by comparing their accumulation in bacterial strains with varying efflux activity. This approach led to the identification of a molecule capable of distinguishing between normal, inactive, and overproducing efflux systems.

After optimization, the method was tested on around 100 *Staphylococcus aureus* isolates previously characterized by conventional techniques, achieving over 80% accuracy. It was also successfully applied to other Gram-positive bacteria. With slight modifications, the method was extended to Gram-negative species such as *Escherichia coli* and *Klebsiella*, with further validation ongoing.

This test is rapid, cost-effective, and easy to perform, delivering results within hours and saving up to 48 hours compared to standard methods. It has been patented and is currently marketed as Colorflux®.

Invited Speaker

Rethinking Clinical Trials for Difficult-to-Treat Resistant Gram-Negative Infections in Europe

Evelina Tacconelli

[1] *Infectious Diseases Center for Translational Research (ID-CARE), University of Verona, Italy, and Chief Scientific Officer, European Clinical Research Alliance on Infectious Diseases (ECRAID)*

Over the past decade, the antimicrobial pipeline has produced several new agents targeting multidrug-resistant Gram-negative pathogens; however, their impact has been limited by the rapid emergence of resistance and heterogeneous activity across resistance mechanisms. Metallo-β-lactamase-producing Enterobacterales and highly resistant Pseudomonas aeruginosa remain challenging targets. Parallel strategies, including microbiome modulation, bacteriophage therapy, and immunotherapeutic approaches, are under investigation and may complement antibiotic therapy, although robust clinical evidence is still evolving. RCT conducted to date have provided important insights but remain limited in their ability to inform optimal clinical management. These studies are typically small, heterogeneous, and primarily designed to support regulatory approval rather than to define treatment strategies for critical ill patients. Consequently, individuals with true "no-option" DTR infections are underrepresented, and the applicability of trial findings to real-world clinical decision-making remains constrained. Guideline development reflects these limitations, with recent recommendations incorporating new antimicrobial agents but often lacking prioritisation and adaptability to local epidemiology, diagnostic capacity, and antimicrobial availability. This has contributed to variability in implementation across regions and highlights the need for more dynamic, evidence-linked clinical algorithms. The presentation will discuss the need to fundamentally rethink clinical trial design. Traditional RCT are poorly suited to the complexity and heterogeneity of DTR infections. Adaptive platform trials offer a more efficient and ethically robust alternative. These designs allow multiple interventions to be evaluated simultaneously and support the continuous evolution of treatment strategies based on accumulating data. Successful advancement in this field will depend on improved data harmonisation, integration of real-world evidence, and the development of perpetual cohort-based platforms. Ultimately, addressing DTR Gram-negative infections requires not only new therapeutics but also a transformation in how clinical evidence is generated, moving from drug-centred evaluation to patient-centred optimisation of treatment strategies in highly vulnerable populations.

Invited Speaker

Bioinspired Antimicrobial Strategy: An Extremophile Deep Sea Peptide to Combat Cystic Fibrosis Infections Caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Aurélie Tasiemski

[1] *Center for Infection and Immunity of Lille, Pasteur Institute, France*

Cystic fibrosis (CF)-associated lung infections caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus* remain difficult to treat due to multidrug resistance and the redox instability of the pulmonary environment, which can impair antibiotic efficacy. In this study, we investigated alvinellacin (ALV), a disulfide-stabilized β-hairpin antimicrobial peptide derived from the deep-sea polychaete *Alvinella pompejana*, as a potential therapeutic agent naturally adapted to redox-fluctuating conditions. The antibacterial and antibiofilm activities of ALV were evaluated against multidrug-resistant clinical isolates under CF-like reducing conditions (6 mM DTT). Circular dichroism analysis showed that DTT did not alter the β-hairpin secondary structure of ALV, supporting its structural stability in CF-like environments. Mechanistic analyses included pore-forming assay, membrane interaction studies, scanning electron microscopy, lipid-binding assays, cytotoxicity testing, and resistance induction assays, while *in vivo* efficacy was assessed using the *Galleria mellonella* infection model. ALV demonstrated strong bactericidal activity that was maintained in the presence of NaCl or human serum. ALV did not induce bacterial resistance and effectively inhibited early-stage biofilm formation and disrupted preformed biofilms, including those of the clinical isolate, even under reducing conditions. The peptide showed selective permeabilization of bacterial membrane linked to its stronger affinity for bacterial membrane lipids and negligible interaction with host-like membranes, with no observed cytotoxicity. *In vivo*, ALV significantly improved survival in infected larvae. These findings highlight ALV as a promising redox-resilient antimicrobial candidate for treating multidrug-resistant CF lung infections.

Invited Speaker

Emerging Antibiotic Resistances Worldwide

Patrice Nordmann

[1] *Molecular and Medical Microbiology Unit, Department of Medicine, University of Fribourg, Switzerland*

Clinically significant multidrug resistance is increasingly reported, particularly among enterobacterial species (e.g., *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp.), as well as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

The antibiotic resistance traits that are already widespread in these Gram-negative bacteria are mainly due to extended-spectrum β-lactamase (ESBL) and carbapenemase production. ESBLs confer resistance to all β-lactams, with the exception of cephamycins and carbapenems, whereas carbapenemases confer resistance to virtually all β-lactams, including carbapenems.

Although novel β-lactams have recently been introduced, such as cefiderocol, ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam, and aztreonam-avibactam, emerging resistance to these agents has already been observed.

In addition, combined resistance to other antibiotic classes, potentially leading to pandrug resistance, may be associated with virulence traits. This recent development may further complicate the future management of infections associated with multidrug-resistant bacteria.

Invited Speaker

Cracking Nature's Recipes to Design Lipid-Targeting Antibiotics

Markus Weingarth

[1] *NMR Spectroscopy Group, Utrecht University, Utrecht, The Netherlands*

Antimicrobial resistance is a global health threat, calling for new antibiotics. Good candidates could be peptide-based compounds that target special lipids that only exist in bacterial, but not in human cell membranes. These drugs kill pathogens without detectable resistance, which has generated considerable interest.

Here, using an integrative structural biology approach, we show that drugs that target special lipids in bacterial membranes use sophisticated supramolecular killing mechanisms[1-3].

1. *Shukla, R. et al. Teixobactin kills bacteria by a two-pronged attack on the cell envelope. Nature 608, 390-396, doi:10.1038/s41586-022-05019-y (2022).*
2. *Shukla, R. et al. An antibiotic from an uncultured bacterium binds to an immutable target. Cell 186, 4059-4073.e4027, doi:10.1016/j.cell.2023.07.038 (2023).*
3. *Jekhmane, S. et al. Host defence peptide plectasin targets bacterial cell wall precursor lipid II by a calcium-sensitive supramolecular mechanism. Nature Microbiology 9, 1778-1791, doi:10.1038/s41564-024-01696-9 (2024).*
4. *Ntallis, C., Martin, N., Edwards, A., Weingarth, M. (2025) Nature Reviews Microbiology, Bacterial cell envelope-targeting antibiotics*

Invited Speaker

Silencing Bacterial Communication With Lactic Acid Bacteria Metabolites: An Eco-Friendly Approach Against Foodborne Pathogenic Biofilms

Georgios Vafeiadis[1], Spyros Didos[1,2], Anagnostis Argiriou[1,2], Efstathios Giaouris[1]

[1] *Department of Food Science and Nutrition, School of the Environment, University of the Aegean, 81400 Myrina, Lemnos, Greece*
[2] *Institute of Applied Biosciences, Centre for Research and Technology Hellas, 57001 Thermi, Thessaloniki, Greece*

Foodborne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus* can persist in food processing environments by forming resilient biofilms that resist standard sanitation methods and serve as long-term sources of contamination. Biofilm growth is often controlled by quorum sensing (QS), including the autoinducer-2 (AI-2) system, which orchestrates communication within and between bacterial species and promotes group behaviors. Targeting QS provides a promising, non-lethal approach for controlling biofilms. Lactic acid bacteria (LAB), commonly used in foods, are known for improving safety and quality, and recent evidence shows their metabolites can interfere with QS and prevent biofilm formation without harming free-floating bacteria, thus lowering the risk of resistance. In this study, we tested a sterile, pH-neutralized (pH 6.5) cell-free supernatant (CFS) from a *Pediococcus acidilactici* cheese isolate for its ability to prevent mixed-culture biofilm formation by three *L. monocytogenes* strains from poultry on stainless steel surfaces. This LAB strain was selected from a larger collection because of its ability to inhibit AI-2 signaling in a *Vibrio harveyi* bioluminescence reporter assay. During biofilm formation, the CFS (prepared in MRS broth) was added at 50% (v/v) to Brain Heart Infusion (BHI) broth and incubated at 37 °C for 48 h under nutrient-matched conditions with and without CFS. Treatment with *P. acidilactici* CFS reduced *L. monocytogenes* biofilm cell counts on stainless steel by approximately 99% (2 logs), while leaving planktonic bacterial populations unaffected. The antibiofilm effect remained stable after heat treatment (100 °C, 5 min), indicating the presence of heat-resistant metabolites. Ongoing work aims to identify these QS-interfering compounds and further examine their effects on AI-2 signaling and biofilm development. Our main goal is to develop environmentally safe, QS-based biocontrol methods that could preserve beneficial microbiota while controlling harmful biofilms in food environments.

Invited Speaker

Are We Measuring or Controlling AMR? Carbapenemases as a One Health Sentinel from Food Systems to Environmental Policy

Luísa Peixe

[1] *Applied Molecular Biosciences Unit (UCIBIO), Faculty of Pharmacy of the University of Porto (FFUP), Portugal*

Antimicrobial resistance (AMR) is driven by the global dissemination of carbapenemase-producing Enterobacterales, compromising last-resort antibiotics and challenging clinical management. Within a One Health framework, carbapenemases represent key molecular markers to investigate transmission dynamics across human, animal, food, and environmental compartments. This presentation integrates current evidence on the occurrence and spread of carbapenemase producers along the food chain, including food-producing animals, retail products, and processing environments. Mechanistic insights into persistence and dissemination remain limited but may involve biofilm formation and horizontal gene transfer. Despite advances in surveillance, critical limitations remain, including inconsistent detection methodologies, underrepresentation of specific resistance mechanisms, and insufficient integration of genomic data for source attribution. Recent environmental monitoring initiatives expand surveillance capacity but frequently lack defined thresholds for intervention and translational pathways to control. By focusing on carbapenemases, this talk evaluates the extent to which current surveillance frameworks inform effective mitigation strategies, emphasizing the need for integrated, risk-based approaches with clear translational impact across the One Health continuum.

Invited Speaker

Antimicrobial Resistance from the One Health Perspective. The Environment in the Equation

Carmen Torres

[1] *University of La Rioja, Faculty of Science and Technology, OneHealth-UR Research Group, Logroño, Spain*

Antimicrobial resistance (AMR) is a global problem that causes great concern to health and scientific authorities and compromises the use of antimicrobial agents in the treatment of infectious diseases. This problem needs to be addressed from the One Health perspective in which human, animal and environmental health are interconnected. Information about the relevance of the environment in this context has been increasingly reported in the last years. The group of Antimicrobial Resistance from de One Health perspective (OneHealth-UR) has analyzed AMR in different environmental niches as is the case of wastewater-treatment plants (WWTP) and wildlife animals, as storks and wild rabbits, among others. Two WWTPs of La Rioja have been followed (influent, effluent, sludges and receiving river at upstream and downstream points) using different technologies, with special emphasis in the dissemination of Extended-Spectrum-beta-Lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates (ESBL-Ec/Kp) and carbapenemase-producing Enterobacterales (CP-E), and results will be shown as well as the comparative analysis with clinical isolates in the surrounding hospital. Moreover, the occurrence of critical mechanisms of resistance linked to ESBL-Ec/Kp and CP-E in wild birds or *mecC*-mediated methicillin-resistant *Staphylococcus aureus* in wild rabbits will be also reported through results obtained in our group in comparison with those of other groups. All these data will allow to increase our knowledge about the relevance of the environment as a crucial link in the study of AMR at global level, to adopt the adequate decisions to manage and control the AMR crisis.

Invited Speaker

Real-World Insights: Breaking Resistance to Amoxicillin–Clavulanic Acid with 1,8-Cineole

Adnane Remmal

[1] *Faculty of Science, Sidi Mohamed Ben Abdellah University, Morocco*

The combination of amoxicillin (AMX) and clavulanic acid (CA) remains one of the most widely prescribed antibiotic therapies. However, its clinical efficacy is increasingly compromised by the emergence of bacterial resistance and persistence mechanisms, among both Gram-negative and Gram-positive pathogens.

To address this limitation, we developed a novel formulation combining AMX and CA with 1,8-cineole (CN), a natural compound well recognized in the pharmacopoeia for its broad pharmacological activities and favorable safety profile.

The obtained in vitro, in vivo and clinical trials demonstrate that this triple combination significantly enhances the antibacterial activity of AMX/CA against susceptible strains. Importantly, it also restores susceptibility in resistant and persistent bacterial populations.

In parallel, in silico investigations were conducted to elucidate the underlying mechanisms of action. Key bacterial targets were explored, including penicillin-binding proteins (PBPs), as well as molecular targets associated with bacterial persistence. These computational analyses suggest that AMX, CA, and CN can form multiple molecular complexes, including three binary combinations (AMX–CA, AMX–CN, and CA–CN) as well as a ternary complex (AMX–CA–CN). These entities may act simultaneously on bacterial targets and significantly reduce the probability of resistance emergence and bacterial survival in a persistent state.

Invited Speaker

Why Are There Virtually No Antibiotics in Clinical Use Based on Bioactive Compounds From Plants?

Walter Luyten

[1] *Department of Biology, KU Leuven, Belgium*

Infections are often treated with (medicinal) plants by traditional healers. Extracts from these (as well as many other) plants often exhibit antimicrobial activity in vitro. In fact, numerous bioactive compounds with antimicrobial activity have been isolated and identified from such extracts. However, very few of these compounds have been developed into clinically used antibiotics. This contrasts strongly with antimicrobial compounds from microorganisms.

Various reasons for this apparent paradox have been proposed. These include (1) insufficient potency of most antimicrobial natural products, (2) synergy required with other plant secondary metabolites for full efficacy, (3) insufficiently chemical or in vivo stability, (4) low oral bioavailability, (5) unfavorable pharmacokinetics, (6) toxicity or small therapeutic window, (7) narrow antimicrobial spectrum, (8) rapid development of resistance, (9) difficult to patent, (10) no economically viable production possible (by full synthesis, semi-synthesis, bioproduction, etc.). In this talk, I will review and evaluate each of these, and draw some conclusions that may guide future research in this area.

S1. Antimicrobial Resistance Mechanisms

Potential Efflux Pump Inhibition by the Cyclic Peptide MV6 Restores Netilmicin Activity in *Acinetobacter baumannii*

Roger de Pedro-Jové[1, 2], Jimmy Lucas[1], Natalia Roson-Calero[1, 3], Pol Oliveras Julia[1, 2, 3], Elisabet Guiral[1, 3], Clara Ballesté-Delpierre[1, 2, 4], Jordi Vila[1, 2, 3, 4]

[1] *ISGlobal, Barcelona Institute for Global Health, Barcelona, Spain*
[2] *CIBER de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain*
[3] *Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain*
[4] *Department of Clinical Microbiology, Biomedical Diagnostic Center (CDB), Hospital Clínic de Barcelona, Barcelona, Spain*

Introduction
Acinetobacter baumannii is a critical global health threat due to its opportunistic nature and ability to evolve multidrug resistance. To combat the lack of effective treatments, adjuvants such as the cyclic peptide MV6 are being explored. While MV6 lacks intrinsic antimicrobial activity, it enhances the activity of aminoglycosides such as netilmicin. This project aims to elucidate the mechanism by which MV6 potentiates the activity of netilmicin.

Methods
Spontaneous mutants were generated under selective pressure from netilmicin alone or in combination with MV6. Whole-genome sequencing (WGS) and a custom variant-calling pipeline identified the mutational distribution. A representative subset of mutants, with the wild-type reference, underwent transcriptomic analysis. To validate the role of efflux systems, Minimum Inhibitory Concentrations (MICs) were determined in the presence and absence of the efflux pump inhibitor PaβN.

Results
WGS and variant-calling analysis revealed a convergent mutational profile across both spontaneous mutant groups; no differences were detected between mutants generated with or without MV6. Key mutations were identified in a tetR-family transcriptional regulator located in cis to a multidrug efflux pump, alongside a recurrent deletion in the intergenic region downstream of an adeABC operon. Additionally, diverse mutations were detected in the coding sequence or promoter region of an ATP-binding protein. Remarkably, transcriptomic analysis confirmed that these mutations resulted in the overexpression of the adeABC operon and the multidrug efflux pump. Preliminary phenotypic assays with PaβN corroborated that, as efflux inhibition partially restored netilmicin susceptibility, efflux systems are the primary driver of resistance.

Conclusion
Our results demonstrate that netilmicin resistance is primarily driven by overexpression of efflux systems. Interestingly, MV6 did not alter the mutational or transcriptomic profile of the mutants. This suggests that MV6 enhances netilmicin without exerting additional selective pressure, supporting its potential role as a “silent” adjuvant that may interfere with efflux function.

Investigations Of Delafloxacin Resistance Mechanisms in *Escherichia coli* and *Klebsiella pneumoniae* Clinical Isolates

Bela Kocsis[1], András Kubicskó[2], Dániel Gulyás[3], Katalin Kamotsay[4], Dóra Szabó[1, 5, 6]

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Delafloxacin is a new fluoroquinolone agent that has been introduced into clinical application. In this study, efficacy of delafloxcin on clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* was evaluated. Altogether, 80 *E. coli* and 43 *K. pneumoniae* were included in this study in Budapest, Hungary. Antimicrobial susceptibility testing was performed by broth microdilution for ciprofloxacin, delafloxacin, levofloxacin, moxifloxacin, ceftazidime, cefotaxime, and imipenem. Selected multidrug-resistant and delafloxacin resistant strains were further analyzed by whole-genome sequencing (WGS) on Illumina MiSeq platform to detect resistance determinants and sequence types (ST). Based on susceptibility results of *E. coli,* delafloxacin and ciprofloxacin resistance rates were 38.75% (31/80) and 41.25% (33/80), respectively. In the case of *K. pneumoniae,* delafloxacin and ciprofloxacin resistance rates were 46.5% (20/43) and 51% (22/43), respectively. WGS analysis detected mutations of quinolone resistance determining regions (QRDR) in selected delafloxacin resistant *E. coli* strains; namely, mutations in *gyrA* Ser83Leu, Asp87Asn and *parC* Ser80Ile were detected in ST162, ST57 and ST15840 strains. In the case of ST131 strains, five combined mutations were detected: *gyrA* Ser83Leu, Asp87Asn, *parC* Ser80Ile, Glu84Val, and *parE* Ile549Leu. In ST43 strains, *gyrA* S83L, D87N, *parC* S80I, and E84V mutations were detected. In delafloxacin-resistant *K. pneumoniae, gyrA* Ser83Ile and *parC* Ser80Ile mutations were detected in ST307 and ST147 strains. In the ST377 strain, *gyrA* Ser83Tyr, Asp87Ala, and *parC* Ser80Ile mutations were detected. Each delafloxacin-resistant *K. pneumoniae* strain carried OqxAB and AcrAB efflux pumps. Delafloxacin demonstrates efficacy against certain populations of *E. coli* and *K. pneumoniae*. However, multiple QRDR mutations can confer delafloxacin resistance in *E. coli* and *K. pneumoniae* clinical isolates.

Antibiotic Resistance in Enterotoxigenic and Enteroaggregative *E. coli* Causing Traveler's Diarrhoea: A Genotypic and Phenotypic Correlation Study

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Traveler's diarrhea (TD) remains one of the most prevalent health concerns affecting individuals visiting low- and middle-income countries. TD is mainly caused by infectious agents, particularly enteroaggregative *E. coli* (EAEC) and enterotoxigenic *E. coli* (ETEC). The aim of this study is to characterize antibiotic resistance patterns and underlying resistance mechanisms in EAEC and ETEC isolates causing TD (2018-2023).

A total of 46 ETEC and 44 EAEC strains isolated from TD patients attending Hospital Clínic (Barcelona) were analyzed. Antibiotic resistance profiles were determined using phenotypic methods based on minimum inhibitory concentration (MIC) and genotypic analysis by whole-genome sequencing using Illumina technology.

Phenotypic resistance patterns were similar between pathotypes, except for levofloxacin (LEV), ciprofloxacin (CIP) and ceftriaxone (CRO), for which ETEC showed higher resistance rates. Among EAEC isolates, resistance rates were: cefepime (FEP) 9.1%, cefazolin (FAZ) 50%, ceftazidime (TAZ) 13.6%, CRO 11.4%, ampicillin (AMP) 52.3%, aztreonam (AZT) 18.2%, LEV 2.3%, CIP 2.3%, and trimethoprim/sulfamethoxazole (SXT) 61.4%. In ETEC isolates, resistance rates were: FEP 17.4%, FAZ 65.2%, TAZ 17.4%, CRO 21.7%, AMP 52.2%, AZT 19.6%, LEV 13.3%, CIP 13%, and SXT 43.5%. All phenotypically resistant isolates showed corresponding genotypic resistance mechanisms. For β-lactams, the most frequent mechanisms were TEM-1 (n=32) and CTX-M-15 (n=13). The *dfrA* gene was identified in SXT-resistant isolates, while mutations in *gyrA* and *parC* were responsible for LEV and CIP resistance. Isolates acquired in Asia showed the highest overall resistance rates, whereas SXT resistance was more frequent in isolates from Africa.

The dissemination of multidrug-resistant EAEC and ETEC through international travel represents a significant risk for the spread of antimicrobial resistance, both through the introduction of multidrug-resistant strains and via the dissemination of resistance genes carried on mobile genetic elements. This risk appears to be elevated in Asia and Africa, highlighting the need for continuous surveillance and antimicrobial stewardship in TD management.

Epidemiological Features of Catheter-Associated Bloodstream Infections in the Neonatal Intensive Care Unit of a Category III Maternity Hospital in Armenia

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Background: Catheter-associated bloodstream infections (CABSIs) represent a major challenge in neonatal intensive care units (NICUs), particularly among preterm infants requiring invasive procedures. Umbilical and peripheral venous catheters are indispensable in neonatal care but pose significant infection risks.

Methods: A microbiological study was conducted between 2020 and 2023 at the Research Centre for Maternal and Child Health Care in Yerevan, Armenia. Catheters removed from 109 neonates admitted to a category 3 NICU were examined to determine microbial growth and antimicrobial resistance. Demographic, clinical, and microbiological data were analyzed using descriptive statistics and chi-squared tests.

Results: The mean gestational age of neonates was 31 weeks; 60% were born preterm, with 23% extremely low birth weight (1000 g). Catheters were in place for an average of 32 hours. Microbial growth was identified in 26 catheters (24%), including 23 with bacterial isolates (21%) and 3 with fungal isolates (3%). The predominant bacterial genera were *Klebsiella spp.* (n=7 (27%)), *Enterobacter spp.* (n = 6 (23%)), *Escherichia spp.* and *Staphylococcus spp.* (both with n=4 (15%)). The susceptibility of the 23 bacterial cultures isolated from the catheters was tested against a common set of antibiotics. The discovered bacterial species were most often resistant to Vancomycin and Gentamicin in a pooled analysis (100% and 96%, respectively). Conversely, Meropenem and Ciprofloxacin showed the least resistance rate (30% and 17%, respectively).

Conclusion: This study underscores the importance of microbiological surveillance in NICUs to better understand the etiology and resistance profiles of pathogens associated with CABSIs. By refining our approach to catheter management and infection control, we can enhance the safety and outcomes for neonates requiring invasive procedures. Future research should focus on improving catheter care protocols and investigating interventions that may reduce the incidence of infections in this high-risk group.

S2/3. Antimicrobials, Antimicrobial Resistance, Ethnopharmacology and One Health

The Impact of Novel Antimicrobial Three-Dimensional Printing (3DP) Dentures on Salivary Biofilm Formation: An *In Vitro* Study.

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Denture-associated biofilms remain a significant cause of oral infections such as denture stomatitis. This study evaluated the antibiofilm efficacy of a newly developed silver-based antimicrobial denture material fabricated via stereolithography and compared its performance with that of three-dimensional printed (3DP) methacrylate dentures without an antimicrobial additive (Control). Rectangular test specimens (7.5 cm × 2.5 cm) were printed using stereolithography. The experimental denture material incorporated sodium silver zirconia hydrogen phosphate (SSZHP) as an antimicrobial additive (GL3D), while the control group comprised non-antimicrobial dentures. Both materials were tested in a mouth-simulated drip-flow biofilm reactor inoculated with pooled human saliva and enriched with clinically relevant oral microorganisms, including *Candida albicans*, *Staphylococcus aureus*, Streptococcus spp., methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant enterococci (VRE). The biofilm's growth and testing were carried out in accordance with a modified version of ASTM E2647-24 protocol. Microbial attachment was quantified on agar media by colony-forming unit (CFU) enumeration. Biofilm architectures were analyzed by confocal laser scanning microscopy (CLSM). Two-way ANOVA was performed using Minitab 2. The log CFU counts in GL3D were consistently lower than those in control (p 0.05) across all microbial groups tested. CLSM analysis showed a 75.37% decrease in biofilm volume, a 49.02% reduction in mean biovolume, a 93.78% decrease in biomass, a 79.03% drop in substratum coverage, and a 92.37% decline in average thickness compared to controls. The incorporation of SSZHP into a 3D-printed denture material markedly reduced microbial viability and disrupted biofilm formation and structure. These findings demonstrate the potential of silver-based 3D-printed denture materials to improve oral health by minimizing biofilm-associated infections and enhancing the hygiene and longevity of prosthetic devices.

Occurrence and Removal of Antibiotics and Antibiotic Resistance Genes in Urban Wastewater Treatment Plants: A One Health Perspective

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Urban wastewater treatment plants (WWTPs) are increasingly recognized as critical control points in the environmental dissemination of antibiotics and antibiotic resistance genes (ARGs), playing a central role within the One Health framework. If insufficient removal of antibiotics or ARGs occurs during wastewater treatment, it may facilitate the spread of antimicrobial resistance (AMR), underscoring the need to assess treatment performance.

This study investigated the occurrence, concentration, and removal of selected ARGs and antibiotics in two urban WWTPs in southeastern Spain. The plants differed in their treatment schemes, with one conducting conventional secondary treatment and the other incorporating tertiary treatment; both included lagooning systems as additional stages. A total of nine ARGs associated with resistance to clinically relevant antibiotics were analysed, *intI1, sul1, mcr-1, blaKPC-3, blaTEM, blaCTX-M, blaCTX-M-32, blaOXA-48,* and *blaOXA-58*, together with the bacterial marker gene 16S rRNA. ARG concentrations were quantified by quantitative polymerase chain reaction (qPCR) and expressed as $\log_{10}$ absolute gene copies. In parallel, selected antibiotics were quantified using high-performance liquid chromatography.

The results showed substantial reduction of both antibiotics and most ARGs in the final effluents, indicating that the applied treatment configurations, including lagooning, effectively reduce the environmental load of AMR. This reduction is particularly relevant from a One Health perspective, as it limits the dissemination of resistant bacteria and determinants from urban wastewater into receiving aquatic environments and water reuse systems. However, certain genes of high clinical and epidemiological relevance, such as intI1, mcr-1, and blaOXA-48, showed limited removal or, in some cases, higher concentrations in effluents than in influents. Correlation analyses revealed relationships between antibiotic concentrations and ARG abundances, providing insight into interactions driving resistance dissemination. This behaviour may reflect complex microbial and physicochemical processes during treatment and highlights the need to better understand ARG dynamics, reinforcing the role of WWTPs as key monitoring interfaces.

New Insights into the Action of Natural Lipoglycopeptides: Gausemycins

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The bacterial cytoplasmic membrane is a compelling target for novel antibiotics due to its essential nature and relative genetic stability. Agents disrupting membrane integrity are less prone to developing conventional resistance, remain effective against metabolically dormant persister cells and biofilms, and employ rapid, diverse bactericidal mechanisms. Natural products have consistently proven to be a fertile ground for drug discovery, yielding unique molecular scaffolds with therapeutic potential (Butler et al., *J. Nat. Prod.*, 2025). A prominent class among them is natural antimicrobial peptides (AMPs), which often operate via membrane-associated mechanisms. Within this promising landscape, our research focuses on the gausemycins—a unique family of Ca^{2+}-dependent (glyco)lipopeptide antibiotics that exhibit activity against Gram-positive pathogens (Tyurin et al., *Angew. Chem.* 2021; Kravchenko et al., *J. Nat. Prod.* 2024). However, the precise details of their membrane interaction and subsequent bactericidal mechanism remain to be fully elucidated. This study aims to define these molecular details and evaluate their specific interplay with key membrane components.

Our approach combines kinetic analysis of bactericidal activity with studies using model lipid membranes to define the membrane-disrupting properties of gausemycins. To validate the membrane as the primary target and probe resistance pathways, we characterized spontaneous resistant mutants and assessed activity against bacterial strains with engineered membrane alterations, including *Bacillus subtilis* overexpressing the ugtP gene and clinically relevant daptomycin-resistant strains with lysyl-phosphatidylglycerol-enriched membranes. Finally, fluorescence microscopy was utilized to directly visualize the cellular localization of gausemycin, providing direct evidence for its membrane-targeting mechanism.

This work unravels the mechanism of gausemycin action, positioning it as a distinct class of membrane-targeting antibiotics. The established combination of membrane association and bactericidal efficacy highlights their potential as a promising new class of antibiotics.

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Antimicrobial Peptides SET-M33L and SET-M33L-PEG Are Promising Agents Against Strong Biofilm-Forming Clinical Strains of *P. aeruginosa*, Including Multidrug-Resistant Isolates

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Background: Biofilm formation by multidrug resistant (MDR) *Pseudomonas aeruginosa* contributes to increased morbidity and mortality in patients with pulmonary diseases. SET-M33L is a synthetic tetra-branched peptide that previously showed antimicrobial activity on standard strains of MDR gram-negative bacteria and improved stability in biological fluids than linear versions. PEGylated version of the peptide, SET-M33L-PEG, further displayed enhanced resistance to *P. aeruginosa* elastase. Therefore, we investigated these two antimicrobial peptides (AMPs) for their antimicrobial and antibiofilm activity against 10 selected *P. aeruginosa* clinical isolates, including MDR strains.

Methods: The effect of AMPs on outer membrane (OM) integrity was investigated by measuring N-Phenyl-1-naphthylamine (NPN) uptake. Their minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs), minimum biofilm inhibitory concentrations (MBICs) were evaluated with gold standard methodologies. Effect on pre-formed biofilm was evaluated measuring bacterial viability assessed by resazurin dye assay. Conventional antimicrobial compounds tobramycin, ceftazidime, and polymyxin B were used as comparator controls.

Results: Dose-dependent increase of NPN fluorescence intensity after treatment with AMPs indicated a disruption of OM integrity in all tested strains. MICs and MBCs values of the AMPs were 7 to 100 times lower than for tobramycin, and 10 to 300 times lower than for ceftazidime, for the respective MDR strains. AMPs MBICs values ranged from 0.3 μM to 21.8 μM, while tobramycin presented the highest MBIC values among the compounds, ranging between 2.1 μM and 273.8 μM. With increasing concentrations of AMPs, a reduction in cell viability of 50% to 100% was observed in pre-formed biofilms. Fractional inhibitory concentration (FIC) indices showed an additive effect (0.5 FBC 1), while fractional bactericidal concentration (FBC) indices showed synergistic effects (FBC 0.5) for most isolates when the AMPs were combined with tobramycin or ceftazidime.

Conclusion: SET-M33L and SET-M33L-PEG are promising antimicrobial agents against strong biofilm-forming *P. aeruginosa*, including MDR isolates.

Genes on the Move: Exploring Microbiomes within Aquatic Ecosystems of First Nation Communities in Manitoba

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Oxidation lagoons (OLs) are widely used wastewater treatment systems in rural and First Nation (FN) communities in Canada and are essential for protecting public health. Although designed to remove pollutants and pathogens, OLs may facilitate the exchange of mobile genetic elements and antibiotic resistance genes (ARGs), promoting antimicrobial resistance (AMR) among environmental bacteria and bacteriophages. This study investigated AMR dynamics by characterizing the resistome of bacterial isolates and metagenomic samples from an OL serving an FN community in Manitoba.
We combined wet- and dry-lab approaches to examine viable bacteria and bacterial and phage metagenomes across treatment stages. Thirty-five samples were collected between September 2022 and April 2023 from raw sewage (RS), lagoon (LG), a submerged attached growth reactor (SAGR), UV-disinfected effluent (EF), and an upstream control site (UPS) including surface water and sediment. Genomic and metagenomic DNA were sequenced using Oxford Nanopore Technology with de novo assembly. Viable isolates were enumerated, and bacterial and Escherichia coli counts were quantified using digital PCR.

Among 58 isolates, Aeromonas (50.87%) and Serratia (15.78%) predominated, with a lower representation of Pantoea, Escherichia, Lelliottia, Rahnella, Enterobacter, Buttiauxella, Acinetobacter, Yersinia, and Citrobacter. ARG analysis identified 32,559 ARG-associated elements, including 425 strict or perfect hits in 84.5% of isolates. Genes conferring resistance to carbapenems, β-lactams, macrolides, tetracyclines, and fluoroquinolones were detected. An Acinetobacter radioresistens isolate from UV-treated effluent carried blaOXA-23 and showed resistance to imipenem (64 µg/mL). Metagenomics (84 samples) revealed Caudoviricetes as the dominant viral class, with aminoglycoside, fluoroquinolone, and phenicol resistance genes most abundant. Gene copy numbers were highest at UPS and RS (1.48 and 1.10 × 10^5 cells/mL). E. coli averaged 172.5 cells/mL in RS.

These findings demonstrate the persistence of clinically relevant bacteria and ARGs in treated wastewater, supporting the need for AMR surveillance in decentralized systems serving FN communities.

Antifungal and Antivirulence Activity of Antimicrobial Peptide Oreoch-1 Against *Candida albicans*

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Objectives: The emergence of drug-resistant fungal pathogens, such as *Candida albicans* (*C. albicans*), has intensified the global demand for antifungal agents with novel mechanisms of action. Conventional therapies, including azoles and polyenes, are increasingly limited by resistance and biofilm-associated tolerance. Antimicrobial peptides (AMPs), naturally expressed in a variety of organisms as first-line defences against microbial infections, have emerged as promising alternatives due to their low propensity for resistance development, ability to disrupt microbial membranes, and inhibition of key virulence traits. While most current studies focus on peptides with antibacterial activity, relatively little is known about their antifungal properties. In this study, we investigated the antifungal and antivirulence activity of the AMP oreochromicin-1 (oreoch-1), isolated from the gills of Nile tilapia (*Oreochromis niloticus*), against *C. albicans*.

Methods: An antifungal susceptibility assay was conducted to determine oreoch-1's minimum inhibitory concentration (MIC), and its effect on growth kinetics was assessed. Hyphal formation was evaluated under filamentation-inducing conditions. Biofilm biomass reduction was quantified using crystal violet staining, and morphological changes were examined microscopically. The molecular responses to oreoch-1 exposure were analysed by qRT-PCR for two virulence-associated genes: *HWP1* (adhesion) and *ERG11* (ergosterol biosynthesis).

Results: Oreoch-1 exhibited potent inhibitory activity with an MIC_{90} of 25 μM, accompanied by reduced growth kinetics, a 67% reduction in biofilm formation, and an 85% reduction in hyphal formation. Microscopic analyses revealed pronounced morphological changes, including membrane disruption, surface deformation, and loss of structural integrity. Oreoch-1 induced a clear dose-dependent downregulation of both *HWP1* and *ERG11*, with maximal suppression observed at MIC_{90}.

Conclusions: Collectively, these findings demonstrate that oreoch-1 targets both structural and regulatory components of *C. albicans* pathogenicity. Its combined effects on growth inhibition, biofilm reduction, hyphal suppression, membrane disruption, and downregulation of virulence-associated genes highlight oreoch-1 as a promising antifungal candidate for the management of *C. albicans* infections.

More Than a Prescription: Behavioural Drivers of Antimicrobial Use in Companion Animals

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Antimicrobial resistance (AMR) represents a major global health challenge requiring coordinated action across human and animal sectors. While antimicrobial use (AMU) in livestock has been extensively studied, prescribing and treatment practices in companion animals remain comparatively underexamined despite the potential for resistant organisms to circulate within shared household environments. Understanding the drivers of antimicrobial exposure in pets is therefore critical for advancing stewardship efforts.

This study evaluated patterns of AMU in dogs and identified behavioural factors influencing treatment decisions among veterinarians and dog owners in Northern Ireland. A behavioural economics framework was applied to explore how time-related decision preferences, particularly present bias, may contribute to antimicrobial demand.

Questionnaire data from 355 dog owners were analysed to characterise prescribing trends and determine predictors associated with antimicrobial exposure. Overall, 58% of dogs had received antimicrobials at least once (mean treatment duration 6 days, SD = 2.06). Amoxicillin-clavulanic acid and metronidazole were the most frequently reported agents, commonly prescribed for skin or wound infections (25%) and gastrointestinal conditions (18%). Logistic regression demonstrated higher odds of antimicrobial use among older dogs (≥8 years; $\beta = 0.11$, $p = 0.002$), insured animals ($\beta = 0.61$, $p = 0.011$), and owners exhibiting present-biased decision-making ($\beta = 0.52$, $p = 0.048$). Owner demographics and household characteristics were not significantly associated with AMU. Although familiarity with AMR was limited (26%), most respondents reported strong trust in veterinary guidance (87%).

These findings suggest that behavioural influences play an important role in antimicrobial exposure in companion animals. Stewardship strategies that align with short-term decision priorities such as emphasising active management plans, structured follow-up, and clear clinical thresholds may help reduce precautionary prescribing. Strengthening behaviourally informed communication within veterinary practice could support more judicious antimicrobial use and contribute to broader One Health AMR mitigation efforts.

Participatory Interventions to Reduce Antibiotic Misuse on Small- and Medium-Scale Laying Hen Farms in Peru

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The global increase in antibiotic use in food animals has raised awareness of the need to implement interventions to reduce antibiotic misuse. In Peru, small-scale farmers often have limited knowledge about the proper use of antibiotics and tend to use them incorrectly. Bottom-up interventions and participatory approaches can promote sustainable and long-lasting behavioral changes by valuing farmers' expertise and increasing program acceptability. We aimed to evaluate if participatory interventions can reduce antibiotic misuse on small- and medium-scale laying hen farms from the Ica and Lima regions in Peru. Considering previous surveys and interviews with farmers from the same area, and similar published evidence, the intervention was designed in collaboration with farmers through a prioritization workshop. Prioritized practices included acidification of feed or water, disposal of animal feces, hand hygiene, facility disinfection, and the use of exclusive farm clothing and boots. Intervention farms received training, veterinary advice and a kit consisting of infographics, logbooks, and a container for disposing of antibiotic packaging. Egg production, feed consumption, health incidents and antibiotic use were recorded over a six-month period. A total of 27 intervention farms and 24 control farms were enrolled. During the monitoring period, four farms withdrew from the intervention group and five withdrew from the control group. At baseline, the use of antibiotics as growth promoters was reported by 86.9% (20/23) of intervention farms and 73.7% (14/19) of control farms. Over the intervention period, most practices improved among intervention farms, except for exclusive clothing and boots, while control farms remained largely unchanged. By the end of the study, antibiotic use as a growth promoter remained high (91.3% intervention, 84.2% control). Additionally, other antibiotic misuse—including prophylaxis, metaphylaxis, and treatment of non-bacterial episodes—remained similar between groups (69.6% intervention, 63.2% control), suggesting that participatory interventions alone were insufficient to reduce antibiotic misuse. Quantitative antibiotic use will be estimated using weight-based indicators.

The Impact of Sewer Biofilms on Antibiotic Stability and Antimicrobial Resistance Signals in Wastewater

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Introduction
Wastewater-based epidemiology (WBE) relies on the assumption that antibiotics and antimicrobial resistance (AMR) markers remain stable during sewer transport, allowing wastewater measurements to reflect community-level antibiotic consumption and resistance trends. However, evidence suggests that in-sewer processes, largely linked to biofilm activity, can cause antibiotic degradation and may affect the distribution of antimicrobial resistance genes (ARGs). This creates uncertainty as to whether wastewater signals represent population-level trends or whether they are influenced by in-sewer changes. To address this, we investigated the stability and behaviour of 11 antibiotics and 54 ARGs in laboratory-scale sewer pipes (operated for > 12 months prior to sampling), using influent wastewater from the North-East of Scotland. Clarithromycin and its associated resistance gene ermB_1 were examined in detail.

Methods
Four pipes (clay and plastic, with and without biofilms) were operated in triplicate. Wastewater was sampled over 24 hours without disturbing biofilms. Antibiotics were quantified using direct injection UPLC-MS/MS, while ARG behaviour was assessed using SmartChip high-throughput qPCR.

Results
Biofilm pipes showed greater antibiotic degradation than biofilm-free controls, with clarithromycin degradation reaching 59–65% over 24 hours compared to 20–23% in controls, confirming a biofilm-driven effect on degradation. This suggests that estimation of community antibiotic usage using WBE may require corrections for in-sewer losses. Despite this, the abundance of ermB_1 did not increase in the wastewater. Instead, biofilm systems exhibited equal or lower ermB_1 levels than biofilm-free pipes, with plastic biofilm pipes showing both the highest antibiotic degradation and the lowest ARG signal. This indicates that tracing ARG abundance using WBE may be better suited to assessing long-term trends rather than short-term changes.

Conclusions
These findings demonstrate that sewer biofilms influence antibiotic stability without necessarily promoting increased ARG abundance in wastewater. This supports more robust interpretation of WBE data across multiple antibiotics and resistance genes, improving confidence in wastewater-based AMR surveillance.

A One Health Perspective on *Helicobacter pylori* along the Bogotá River Basin: Environmental and Clinical Genotyping Reveals Virulence Profiles and Clarithromycin Resistance

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Helicobacter pylori is a major human pathogen and a type I carcinogen, the transmission dynamics of which remain incompletely understood. From a One Health perspective, the interaction between environmental reservoirs, human hosts, and antimicrobial resistance represents a major concern. The Bogotá River basin, impacted by pollution and used extensively by communities, constitutes an ecological interface for pathogen circulation and genetic exchange.

Objective: The aim of this study was to characterize the environmental and clinical molecular profiles of *H. pylori* in the Bogotá River basin, focusing on virulence genotypes and clarithromycin resistance, and to assess the potential role of contaminated water in shaping bacterial diversity and antimicrobial susceptibility.

Methods: Fifty-one water samples from different sections of the Bogotá River and 24 gastric biopsies from exposed residents were analyzed. Detection of *H. pylori* was performed by PCR, targeting the *vacA* gene. Virulence genotyping included *vacA* alleles (s, m, i), *cagA*, and *glmM*. Clarithromycin resistance was evaluated by sequencing the 23S rRNA gene to identify resistance-associated mutations.

Results: *H. pylori* DNA was detected in 41.2% of river water samples and 50% of gastric biopsies, demonstrating dissemination. River samples displayed highly heterogeneous virulence genotypes, dominated by *glmM*(+) *cagA*(–) *vacA* s1m1 (28.6%) and s1m1i1 (19%). No water sample harbored the *cagA* gene, and viable bacteria could not be cultured, suggesting persistence primarily in non-culturable but potentially infectious states. Critically, all environmental samples exhibited wild-type 23S rRNA sequences, indicating absence of clarithromycin resistance. In contrast, 25% of clinical isolates carried the A2143G mutation, which is a key determinant of clarithromycin resistance.

Conclusions: These findings support a One Health model in which the aquatic environment acts as a reservoir of *H. pylori* genetic material that may contribute to bacterial evolution and antimicrobial resistance in human hosts through natural transformation. The clear divergence in clarithromycin susceptibility between environmental and clinical samples underscores the role of human-associated selective pressures.

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

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

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

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

Tracking AMR Transmission Throughout the Food Chain

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Our project investigates the transmission dynamics of antimicrobial resistance (AMR) across the food chain, focusing on the dissemination of antibiotic resistance genes (ARGs) and resistant bacteria. We integrate whole metagenome sequencing with culture-based approaches to systematically characterize ARG reservoirs in environmental and food matrices and to evaluate potential connectivity between compartments.

Sampling encompasses manure, agricultural soils prior to manure application, post-fertilization soils, crop products, meat products, and dairy fermented products. For each sample type, we perform taxonomic profiling and quantify total ARG abundance and diversity. Metagenome-assembled genomes (MAGs) are reconstructed to identify ARG-carrying bacterial hosts within complex microbial communities and to guide targeted cultivation strategies. In parallel, antibiotic-resistant bacteria are recovered from all matrices using selective and non-selective approaches. Isolates are subjected to phenotypic antibiotic susceptibility testing to establish resistance profiles, followed by whole genome sequencing for detailed characterization of ARG content, genomic context, and associated mobile genetic elements. Comparative genomic analyses are conducted to assess genetic relatedness among isolates and to explore potential links between environmental, primary production, and food-associated compartments.

Preliminary analyses indicate overlapping ARG patterns between manure and fertilized soils, and in some cases similar genotypic contexts in isolates recovered from different matrices. We have also achieved the genomic-guided recovery of enterobacteria with diverse ARGs. However, the primary emphasis of this work lies in the establishment of a robust, integrative methodological framework that combines metagenomics, culturomics, resistance phenotyping, and high-resolution genomics and creates a specific database to facilitate the analysis of our findings.
By applying this coordinated One Health approach, the study provides a comprehensive platform for investigating AMR distribution and resistance profiles across the food chain, supporting future risk assessment and mitigation strategies grounded in harmonized multi-compartment data generation.

Incq Plasmids Facilitate the Integration of Antibiotic Resistance Genes in the Chromosome

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Plasmids are self-replicating pieces of DNA that are particularly prominent in bacterial genomes. In addition to maintenance genes, plasmids typically carry cargo that allows cells to adapt to environmental challenges (such as exposure to antibiotics) or exploit new niches. The reason why these genes are kept in plasmids as opposed to integrating in the chromosome as not well understood, particularly considering the fitness cost of maintaining plasmids, a question known as "the plasmid paradox". Transient integration into the chromosome in the form of ICE (Integrative Conjugative Elements) appears to be part of the answer. Here we looked for other forms of plasmid integration. We collected 10,000+ complete genomes of *E. coli* from a NCBI. We identified MGEs associated with a plasmid origin of replication in the chromosome, with default parameters (95% id and 60% cov) and found 262 origins of replication in chromosomal sequence. Of these, 147 (56%) belonged to the IncQ incompatibility type and between 13 and 27 to IncF (5-10.3%). The high representation of IncQ origins of replication is remarkable. Unlike most plasmids, which replicate via a theta mode of replication, IncQ plasmids replicate by strand displacement, which includes replication initiation machinery for both strands, and limits plasmid size to less than 15 kb. We also found that, despite its size limitations, chromosome-integrated IncQ plasmids carry a significant load of antibiotic resistance genes. In the following cases: APH(3')-Ia, sul2, APH(3'')-Ib, APH(6)-Id, catI, TEM-1, and CTX-M-1, integrated plasmids present between 5 and 7% of the total representation of the corresponding antibiotic resistance gene in the database. Thus, IncQ-mediated integration in the chromosome appears to facilitate the vertical transmission of a significant number of antibiotic resistance genes.

How Bacteria Survive Their Own Antimicrobial Peptides? The DdD Protein Defines a New Safety Mechanism in Leaderless Bacteriocins

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The rapid rise of antimicrobial resistance urges to develop new antimicrobial strategies that fit within a One Health perspective. Leaderless bacteriocins are attractive candidates, but because they are synthesized in their active form inside the cell, they raise a key question: how do producer bacteria protect themselves, and what does this mean for their safe use as antibiotic alternatives? To harness these molecules therapeutically, we need a clear picture of how they are produced and how immunity is ensured in the producing strain.
Enterocin DD14 (EntDD14) is a two-peptide leaderless bacteriocin produced by *Enterococcus faecalis* 14 that shows antibacterial, antiviral and immunomodulatory activities. Yet the molecular basis of self-immunity during active EntDD14 production has remained unclear. Here, we focused on DdD, a protein encoded within the EntDD14 biosynthetic cluster. *In silico* analyses suggested that DdD is a membrane-associated protein, and our genetic and functional data show that it is not required for EntDD14 production or export, but is crucial for self-immunity. Deleting *ddD* dramatically reduced resistance to both intracellularly produced and exogenously added EntDD14, whereas complementation with *ddD* restored full resistance.
By identifying DdD as a new and highly effective immunity factor, our work updates current models of leaderless bacteriocin biology and underline the importance of accessory proteins as protective “safety locks”, providing new insights into the defense mechanisms associated with leaderless bacteriocin production. These findings can be exploited to engineer safer bacteriocin-producing probiotics, limit off-target toxicity, supporting the responsible use of bacteriocins as antibiotic alternatives in One Health strategies against antimicrobial resistance.

Bacillus subtilis-Derived Peptides as Alternative Treatment to Antibiotics in Multidrug-Resistant *Staphylococcus aureus*: A Mechanistic Study

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Multidrug-resistant *Staphylococcus aureus* (MDR *S. aureus*) is a critical global pathogen, causing significant morbidity and mortality in humans and animals. This pathogen's ability to form biofilms shields it from antibiotics and facilitates the transfer of resistance genes, underscoring the urgent need for new anti-biofilm strategies. Leveraging microbial ecology and coevolution, probiotic species like *Bacillus subtilis* are gaining traction as potential therapies due to their ability to reduce *S. aureus* colonization and virulence. In this study, we isolated and screened 1123 *Bacillus* strains from concentrated livestock environments where frequent interactions with *S. aureus* were believed to occur. Subsequent screening identified *B. subtilis* 6D1, a strain with remarkable ability to inhibit biofilm growth, disassemble mature biofilms, and enhance the antibiotic sensitivity of *S. aureus* biofilms via Agr quorum sensing interference. Biochemical and molecular analyses revealed that multiple surfactin isoforms and an uncharacterized peptide were responsible for this activity. Compared to commercial HPLC-grade surfactin, *B. subtilis* 6D1-derived peptides more effectively inhibited biofilm formation across all four *S. aureus* Agr backgrounds and prevented *S. aureus*-induced cytotoxicity in HT29 human intestinal cells. Using adaptive laboratory evolution to understand the effects of long-term exposure to anti-biofilm compounds, we found that constant exposure to *B. subtilis* 6D1 peptides increased biofilm formation when exposure ceased, reduced cytotoxicity, and decreased interspecies competitiveness of *S. aureus.* These results suggest that while anti-biofilm compounds may reduce the likelihood of selecting for antibiotic resistance, they can still drive bacterial adaptation that promotes biofilm production and possible recalcitrance in the absence of these compounds. Our findings highlight the need to anticipate adaptive trade-offs when developing anti-biofilm therapies. Overall, this study illustrates the potential of exploiting microbial diversity to discover novel anti-biofilm agents, offering promising strategies to combat MDR *S. aureus* infections and enhance antibiotic efficacy in clinical and veterinary settings.

The Action of Phytochemical Products in Antibiotic Potentiation and Biofilm Control

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The limitation of our current arsenal of effective antibiotics, together with the lack of new antibacterial alternatives, is accelerating the onset of a “post-antibiotic era” that threatens many achievements of modern medicine. Recycling existing antibiotics represents a promising strategy to address the antimicrobial resistance crisis. In particular, combining antibiotics with non-antibiotic molecules that target specific resistance mechanisms may be more effective than the discovery of entirely new drugs. Natural products, especially phytochemicals derived from plant secondary metabolism, display antimicrobial activity and are therefore of significant interest for antimicrobial therapy.
In this study, a panel of 50 phytochemical products from different chemical classes (alkaloids, glucosinolate hydrolysis products, phenolics, and terpenes) was evaluated for antimicrobial activity and antibiotic potentiation against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Modes of action were investigated by assessing interference with key physiological processes, including motility, extracellular enzyme production, quorum sensing, membrane integrity, and intracellular content release, in order to clarify the mechanisms underlying antibiotic potentiation. Phytochemicals were also assessed for biofilm control, examining effects on bacterial viability and biofilm structure.
Not all phytochemicals exhibited antimicrobial or antibiotic-potentiating activity. Among the most active compounds, furvina showed strong antimicrobial effects against both *P. aeruginosa* and *S. aureus* by disrupting bacterial membranes and inducing intracellular content release. Furvina also potentiated antibiotic activity and inhibited the quorum sensing system of *P. aeruginosa* and related phenotypes, although it showed no activity against the Agr quorum sensing system of *S. aureus*. Phytochemicals that interfered with quorum sensing significantly impaired biofilm formation but had limited effects on established biofilms, particularly regarding biofilm dispersal.
In conclusion, phytochemicals can complement antibiotic activity and effectively prevent biofilm formation, although they are unable to induce dispersal of mature biofilms. This approach supports future Nature-based strategies targeting resistance without increasing selective pressure.

S4. Conventional and Novel Approaches in the Discovery of New Antimicrobial Agents

A Novel Cyclic Peptide CAP-18 and its Enantiomer Cyclic D-CAP-18 as Potential Agents Against Multidrug-Resistant Bacteria

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Background
Antimicrobial peptides (AMPs) represent promising candidates in the search for new antimicrobial agents. The increasing incidence of infections caused by multidrug-resistant (MDR) bacteria, together with the declining efficacy of existing antibiotics, highlights the urgent global need for novel therapeutics. CAP-18 is an α-helical peptide belonging to the cathelicidin family. Its enantiomer, D-CAP-18, also shows potential for drug development and optimization. Because linear peptides are often unstable in vivo, cyclization of both peptides was undertaken to improve stability. This study aimed to evaluate the antimicrobial activity of cyclic CAP-18 and cyclic D-CAP-18 against MDR bacteria.

Methods
Antimicrobial activity was assessed using broth microdilution assays to determine minimum inhibitory concentrations (MICs) of the cyclic peptides against American Type Culture Collection (ATCC) reference strains and a panel of well-characterized clinical MDR isolates, including extended-spectrum β-lactamase (ESBL)- and carbapenemase-producing strains. Four MDR *E. coli* and five MDR *K. pneumoniae* clinical isolates were tested.

Results
Both cyclic CAP-18 and cyclic D-CAP-18 exhibited greater activity against Gram-negative than Gram-positive bacteria. MIC values for cyclic CAP-18 against *E. coli* ranged from 2 to 8 mg/L, while cyclic D-CAP-18 showed MICs of 2 to 4 mg/L. Against *K. pneumoniae*, cyclic CAP-18 demonstrated MICs of 4 to 32 mg/L, and cyclic D-CAP-18 showed MICs of 4 to 16 mg/L.

Conclusion
Cyclic CAP-18 and cyclic D-CAP-18 display promising antimicrobial activity against MDR Gram-negative bacteria, including both ATCC reference strains and clinical isolates. These preliminary findings support their potential as candidates for further antimicrobial drug development.

Efflux-Modulating Antimicrobial Peptide Combinations Restore Ciprofloxacin Activity Against Clinical *Escherichia coli*

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Escherichia coli remains the leading cause of urinary tract infections worldwide, while antimicrobial resistance (AMR) poses a growing public health threat, contributing to over 1.2 million deaths annually in 2019 and projected to cause 10 million deaths per year by 2050. Extensive and often inappropriate use of fluoroquinolones, particularly ciprofloxacin, has accelerated resistance development in E. coli, largely driven by target-site mutations and efflux pump overexpression, thereby severely limiting therapeutic options. Innovative strategies that enhance antibiotic efficacy are therefore urgently required to address this challenge.

In this study, the antibacterial activities of antimicrobial peptides (AMPs)—Batroxicidin (BatxC), Crotalicidin (Ctn), Latarcin-2a (Ltc-2a), and Tat-2—were evaluated individually and in combination with ciprofloxacin against ciprofloxacin-resistant clinical *E. coli* isolates. Minimum inhibitory concentrations (MICs) were determined using the broth microdilution method. Drug–drug interactions were assessed by checkerboard assays and interpreted using the fractional inhibitory concentration index (FICI) according to EUCAST/CLSI criteria. The contribution of efflux mechanisms was examined using the efflux pump inhibitor carbonyl cyanide 3-chlorophenylhydrazone (CCCP). Cytotoxicity was evaluated in HeLa cells using the MTT assay.

Ciprofloxacin combinations with BatxC, Ctn, and Ltc-2a resulted in fourfold reductions in ciprofloxacin MICs with additive interactions, whereas the ciprofloxacin–Tat-2 combination produced a 16-fold MIC reduction, consistent with partial synergy. Notably, triple combinations of ciprofloxacin, AMPs, and CCCP reduced ciprofloxacin MICs to 1 µg/mL (≥64-fold), highlighting efflux inhibition-mediated antibiotic resensitization. Cytotoxicity analysis showed that Ctn exhibited excellent biocompatibility, with ciprofloxacin–Ctn combinations maintaining ≥75% cell viability.

Overall, AMP-based combination therapy represents a promising, mechanism-driven strategy to restore ciprofloxacin activity against resistant *E. coli*. Further in vivo validation and mechanistic studies are required to support clinical translation.

An Application of Cluster Analysis with the Affinity Coefficient and Euclidean Distance to Bacteria Antimicrobial Resistance Profile

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In this work, the hierarchical cluster analysis (HCA) of time series is proposed for monitoring antimicrobial resistance (AMR). The purpose was to develop a new mathematical tool or new applications that could allow the identification of pairs of bacteria/antimicrobial agents that require early intervention or monitoring.
Time series data of resistant bacteria/antimicrobial agent pairs was obtained from the public website of ECDC in 2023.
Variables: Resistant isolate percentage values in Portugal from 2012 to 2021 were obtained. Methods: HCA was performed directly on the time series data using the standard Affinity coefficient and Euclidean distance. The complete linkage was used as the aggregation criteria. Results: *Enterococcus faecium* | Aminopenicillins presents very high resistance (87,96% +/- 3,12 [min-83,66/max-94.44] and *Escherichia coli* |Carbapenems presenting the lowest resistance values (0,18% +/-0,17 [min-0,02/max-0,53]; this is quite curious, since the WHO considers this pair as a critical priority. The HCA with the (1-standard Affinity coefficient) distance showed three different clusters. The first cluster includes *K. pneumoniae* |Carbapenems and *E. coli* |Carbapenems. The third cluster includes *E. faecium* | Vancomycin, *E. faecium*| Gentamicin, *Enterococcus faecalis* | Vancomycin, *Acinetobacter spp*. | Aminoglycosides, Fluoroquinolones, and Carbapenems. The second cluster is a heterogeneous group showing some clusters not analyzed here. The first cluster includes two Enterobacteria considered Critical Priority by the WHO. These bacteria can produce several resistance mechanisms, namely carbapenemase enzymes. The third cluster includes *Acinetobacter spp*. with all antimicrobial agents studied, including Carbapenems, Enterococcus with Vancomycin and Gentamicin. Conclusions: This was the first time that the Affinity coefficient and Euclidean distance were applied to the AMR profile. The standard Affinity coefficient (Silhouette coefficient's (0.8)) presented to be more suitable than Euclidean distance (Silhouette coefficient's value (0.4)) for identifying patterns and trends in resistance values in AMR data because it was shown to be more robust in capturing similar behaviors in resistance values over time and a better cluster compactness and separability.

Metabolic Signatures of Antibiotic Resistance in *Pseudomonas aeruginosa*: A Systems Biology Approach to Rational Adjuvant Therapy Design

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Antimicrobial resistance (AMR) is an increasing threat according to the World Health Organization. *Pseudomonas aeruginosa* is a Gram-negative and opportunistic organism that develops multidrug resistance in several ways, which requires a better understanding of the mechanisms and new and effective solutions to overcome AMR. The transcriptome, a complete set of RNA molecules, provides information about gene expression, regulation, and function, leading to the translation of this information for disease diagnosis, treatment, and drug development and discovery. Integration of transcriptome data and a genome-scale metabolic model (GEM) of the organism is one of the useful strategies to identify and understand metabolic activities related to AMR. The discovery of reporter metabolites (RM) and their specific metabolic pathways may be a powerful method to study metabolic responses and cellular mechanisms of AMR under different conditions, leading to potential therapeutic targets or biomarkers. This study integrates transcriptomic data from 414 drug-resistant clinical isolates with a genome-scale metabolic model (GEM) of *P. aeruginosa* to identify metabolic adaptations under four antibiotic stresses: ceftazidime (CAZ), ciprofloxacin (CIP), meropenem (MEM), and tobramycin (TOB). Differential gene expression (DGE) analysis revealed largely drug-specific responses, with minimal overlap in differentially expressed genes (DEGs) across conditions. Using the Reporter Metabolite (RM) algorithm, metabolites central to the resistance phenotype were identified. Pathway enrichment analysis highlighted antibiotic-specific alterations in metabolic networks, many of which are associated with biofilm formation and virulence. Based on the findings, we propose a novel adjuvant therapy composed of condition-specific metabolites (propionic and acetic acids, L-inositol, glutamine, glutarate, fumarate, and melatonin) to enhance antibiotic efficacy and reduce resistance development. This systems biology approach provides a comprehensive framework for metabolic intervention in AMR pathogens.

Flufenamic Acid Potentiates Host Clearance of *Staphylococcus aureus* via Dual Inhibition of the Agr Quorum-Sensing System and NLRP3 Inflammasome

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The rising threat of antibiotic-resistant *Staphylococcus aureus* necessitates novel therapeutic strategies. This study elucidates the dual mechanism by which the NSAID flufenamic acid (FFA) enhances bacterial killing by immune cells. We identified the quorum-sensing AgrAC two-component system as a key target of FFA in *S. aureus*. Through promoter-reporter assays, EMSA, and mutagenesis, FFA was shown to bind the response regulator AgrA at a novel site (E27) within its regulatory domain, inhibiting virulence gene expression (e.g., α-toxin). This makes FFA the first reported inhibitor targeting this AgrA domain, distinct from existing DNA-binding domain inhibitors.
Concurrently, FFA's immunomodulatory action is essential for its efficacy. In macrophages, FFA inhibited the NLRP3 inflammasome, a process critical for promoting phagosome-mitochondria colocalization and subsequent bactericidal reactive oxygen species (ROS) generation. This NLRP3-dependent mechanism was validated *in vivo*, where FFA lost its effect in *nlrp3*-KO mice. While exhibiting minimal direct antibacterial activity (MIC >400 μM), FFA acted synergistically with gentamicin, significantly reducing bacterial loads *in vivo*.
Thus, FFA represents a promising anti-virulence agent that uniquely combines suppression of bacterial pathogenicity via AgrA inhibition with potentiation of host innate immunity via NLRP3 blockade. This dual, host-pathogen targeting strategy minimizes selective pressure for resistance and supports the therapeutic potential of repurposing FFA, particularly in topical or combination regimens, against staphylococcal infections.

Bioinformatics and Genomic Analysis for the Potential of mRNAs as Antibacterial Drug Targets in the Genome of Macrolide-Resistant Strains of *Streptococcus pneumoniae* Infecting Humans for Novel Antisense Oligonucleotide Design

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Introduction: The macrolide-resistant strains of *Streptococcus pneumoniae* are the latest additions to the WHO's List of Priority Antibiotic-Resistant Bacteria, responsible for over 3 million deaths annually, of which 300,000 are in children under 5 years of age. One of the most promising novel targets for antibacterial drug development is mRNA. The present study employs a rational framework based on four criteria related to mRNA distribution, function, and metabolism, and identifies them as suitable targets for antibacterial drug discovery. It focuses on seven of them, which are found in the *S. pneumoniae genome*: the RNase P, FMN, TPP, PreQ1-II, Purine, ykoK leader, and Glycine riboswitches.

Methods: The selection of targets and the subsequent rational design of ASOs targeting the macrolide-resistant *S. pneumoniae* are based on bioinformatics and genomic studies, including analyses of international databases, Clustal X multiple alignments, selection of appropriate motifs, BLAST searches, and biochemical pathway analyses.

Results: The mRNAs found in *S. pneumoniae* are grouped into four categories: most suitable, very suitable, suitable, and not suitable. Most suitable riboswitches (FMN, TPP, Purine, and $PreQ_{1)}$ regulate essential metabolites, with no alternative biosynthetic pathways or transport. Very suitable riboswitches (ykoK leader) control the critical metabolite's biosynthesis and transport. Suitable riboswitches (Glycine) have alternative biosynthetic pathways and do not control their transport. Five different antisense oligonucleotides (ASO) have been designed to target them.

Conclusions: Gene expression is regulated at the transcriptional or translational level. Using mRNA, we develop innovative strategies to design ASOs that inhibit *S. pneumoniae* growth. Our proprietary protocols for suitability and ASO design enable us to develop novel ASOs (with precise nucleotide sequences for specific binding, modifications that enhance stability and induce RNase H activity, and attached cell-penetrating peptides) targeted to the most suitable mRNAs, with potential as effective antimicrobial therapeutics with growth-inhibitory effects.

In silico design of synthetic peptides inspired by natural peptides scaffolds from a Mediterranean medical plant *Charybdis pancration* (Steinh.) Speta, with predicted activity against relevant pathogens

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Antibiotic resistance is widely recognized as one of the most pressing global health challenges, resulting from the rapid and uncontrolled spread of multidrug-resistant bacterial and fungal pathogens. The progressive loss of effectiveness of conventional antibiotics highlights the urgent need for alternative therapeutic strategies. In this scenario, antimicrobial peptides (AMPs) have attracted increasing attention as promising candidates for the treatment of infectious diseases, due to their broad-spectrum activity, diverse mechanisms of action, and reduced likelihood of inducing resistance compared to conventional antibiotics.

This study investigates the potential of novel synthetic AMPs inspired by natural peptide scaffolds and developed through advanced rational design approaches. Natural peptides from a Mediterranean medical plant *Charybdis pancration* (Steinh.) Speta were used as starting templates for the design of multiple modified peptide sequences employing in silico tools based on artificial intelligence and deep-learning models. These computational strategies enabled the optimization of key physicochemical properties associated with antimicrobial and antibiofilm activity.

The designed peptides were subsequently evaluated using a combination of in silico analyses and in vitro tests to validate their predicted MICs against relevant Gram-negative pathogens (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*) and Gram-positive pathogens (antibiotic-resistant *Staphylococcus aureus*). In particular, the in silico analysis predicted MIC values against *Pseudomonas aeruginosa* ranging from **48.8 to 10.9 µg/mL**.

Overall, this work highlights the value of integrating natural peptide sources with artificial intelligence-based design methodologies for the discovery of new antimicrobial candidates. Such an approach may help to expand the current antimicrobial pipeline and provide innovative solutions to combat the growing threat of antibiotic resistance.

Marine Fungi as a Source of Antibacterial, Antifungal and Antibiofilm Agents

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Introduction: Marine fungi are an underexplored reservoir of bioactive secondary metabolites with significant potential for the development of new therapeutic agents to combat infectious diseases and antimicrobial resistance.

Broad objectives: In this work, the antimicrobial properties of ten marine fungal strains *Trichoderma afroharzianum* (HP58), *Penicillium citrinum* (HP26), *Talaromyces austrocalifornicus* (HPα8), *Penicillium rubens* (HP10), *Tamaricicola* sp. (PN38), *Phaeosphaeriaceae* sp. (PN33), *Clonostachys rosea* (IG119), *Trichoderma* sp. (HP31), *Talaromyces catalonicus* (HP25), and *Fusarium clavus* (HP54) were investigated using crude extracts obtained from cultures grown on different media.

Methods: Chloroform and butanol extracts from cultures grown on malt extract medium (MEA) were evaluated against Gram-positive and Gram-negative bacterial reference strains exhibiting antibiotic resistance, as well as against the yeast *Candida albicans*. Antimicrobial activity was assessed through determination of the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC) and antibiofilm activity.

Results: The preliminary *in vitro* results identified strains *Penicillium citrinum* (HP26), *Talaromyces austrocalifornicus* (HPα8), *Penicillium rubens* (HP10)and *Clonostachys rosea* IG119 as promising producers of antimicrobial secondary metabolites. In particular, *Clonostachys rosea* IG119 showed an interesting ability to inhibit biofim formation of *Staphylococcus aureus* ATCC 25923 with a Biofilm Inhibition Concentration 50% (BIC_{50}) of 22 μg/ml.

Conclusions: Optimization of cultivation parameters could support the isolation of bioactive compounds and enable their detailed chemical and pharmacological characterization. Overall, these findings indicate that marine fungi deserve to be considered an excellent source of antimicrobials.

Genome Mining as a Tool for Identifying Novel Glycopeptide Antibiotics: Insights from Kineomicins

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Glycopeptide antibiotics (GPAs) (vancomycin, teicoplanin, oritavancin, telavancin, dalbavancin) are clinically used to treat severe infections caused by multi-drug-resistant (MDR) Gram-positive pathogens and *Clostridioides difficile*. Most of these antibiotics are natural products or semisynthetic derivatives of natural products, blocking bacterial cell wall biosynthesis by binding to the terminal D-Ala-D-Ala terminus of the peptidoglycan precursor [1]. Recently, genome mining has emerged as a powerful approach for the identification of novel glycopeptide scaffolds and their previously unrecognized producing organisms [2]. In this framework, we screened 600 genomes belonging to the *Pseudonocardiales* order and identified 18 biosynthetic gene clusters (BGCs) predicted to encode previously unknown GPAs [3,4]. One of these molecules, named kineomicins from its producer strain *Actinokineospora auranticolor*, was produced up to an exceptionally high production rate, exceeding 1 g/L in a benchtop bioreactor. The resulting antibiotic complex was microbiologically characterized and the structure of its main congener, KmcB, was elucidated by LC-MS, MS/MS, and NMR spectroscopy, revealing a unique peptide scaffold [4]. Biological and chemical profiling of kineomicins is ongoing, highlighting the potential of this new antibiotic. Parallel studies on two additional putative GPA-producing strains are further validating genome mining as a key strategy in the discovery and development of new GPAs to counteract AMR spread.

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PLP-3: A Novel Bicyclic Peptide Active Against Multidrug-Resistant Bacteria

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Introduction

Antimicrobial resistance (AMR) is a global health crisis and one of the top ten public health threats according to the WHO. In recent decades, few antibiotics have reached the market to tackle infections caused by multidrug-resistant (MDR) bacteria. Thus, new antimicrobials are needed.
This project evaluates the activity of PLP-3, a synthetic antimicrobial peptide designed *in silico* as a Protegrin-1 analogue, against a panel of MDR pathogens.

Methods

The antibacterial activity of PLP-3 was evaluated by determining the Minimum Inhibitory Concentration (MIC) against a clinical collection of MDR strains, including *Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecalis* and *Enterococcus faecium*. Time–kill curves (TKCs) were performed to assess bactericidal activity, and checkerboard assays were used to evaluate synergies with conventional antibiotics. The Minimum Biofilm Inhibitory Concentration (MBIC) and Minimum Biofilm Eradication Concentration (MBEC) were also determined. Finally, PLP-3-resistant mutants were generated and sequenced to elucidate the mechanism of action.

Results

PLP-3 MIC_{50} and MIC_{90} ranged between 1-4mg/L and 1-16mg/L across all Gram-negative and Gram-positive strains tested, respectively. TKC confirmed that PLP-3 is bactericidal at concentrations ≥ 2xMIC after 24h, with viable counts falling below the detection limit. Checkerboard assays showed that most antibiotic combinations were additive, achieving synergy with meropenem against a clinical strain of *A. baumannii*. As for PLP-3 antibiofilm activity, both $MBIC_{50}$ and $MBEC_{50}$ ranged between 4-32mg/L and 64->64mg/L for Gram-negative strains, respectively, and 1-16mg/L and 16->64mg/L for Gram-positive strains. Regarding resistant mutants, variant calling analysis indicated that the prevalent mechanism appears to involve a reduction in surface negative charge to minimize electrostatic interactions with PLP-3.

Conclusion

PLP-3 shows broad-spectrum bactericidal activity against MDR bacteria for planktonic and biofilm-associated cells, which is a promising candidate for the development of new antimicrobials to combat AMR.

Bacteriophage Depolymerases as a Novel Alternative to Antibiotics: Their Promises and Challenges

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The global rise in antibiotic-resistant bacterial infections necessitates innovative strategies beyond conventional antibiotics. Phage-derived depolymerases selectively degrade bacterial capsules and biofilms, weakening pathogen defenses and enhancing immune clearance. This approach offers a highly specific and resistance-sparing intervention. Our study aims to determine whether phage depolymerases can enhance the efficacy of phage therapy, potentially serving as a novel anti-virulence strategy for bacterial infections. A panel of seven lytic *Acinetobacter baumannii* phages, isolated from local sewage samples, and six recombinant depolymerases were employed. The effects of depolymerases on each phage's host range and bacterial growth inhibition were assessed using spot-testing assays and bacterial growth curves. Synergy assays demonstrated that combining varying concentrations (0.8–50 ng/mL) of depolymerases with 10^2–10^8 PFU/mL of phage significantly inhibited the growth of several *A. baumannii* strains. This combination outperformed high doses of either phage or depolymerase alone, as shown by area under the curve analysis, achieving up to 85% growth inhibition compared to the controls. Notably, certain phage–depolymerase combinations led to a remarkable six-log increase in the phage endpoint titer. Efficiency of plating studies further showed that depolymerases expanded the phage host range, enabling broader therapeutic applications without the need for phage adaptation or engineering. To our knowledge, this is the first study to show that phage depolymerases enhance both the efficacy and host range of therapeutic phages. The promises and challenges of exploring phage depolymerases as a strategy to combat antimicrobial resistance, particularly against bacteria that produce protective capsules or biofilms, will be discussed.

Overcoming Resistance Mechanisms via Membrane Disruption with Newly Developed Antimicrobial Peptides

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The rising threat of antimicrobial resistance makes novel therapeutics necessary. In this study, we designed nature-inspired antimicrobial peptides (AMPs) and evaluated their biological potency and safety profile. The synthesized peptides exhibited exceptional antibacterial and antifungal activity, characterized by low minimum inhibitory concentration (MIC range: 0.5-2 µg/mL), values across all standard ATCC strains tested (*E. coli*, *S. aureus, P. aureginosa* and *C.albicans*). Meanwhile, cytotoxicity assays confirmed their selectivity as they displayed no significant toxic effects on mammalian cells. Building on this favorable safety index, the peptides also demonstrated high stability against proteolytic degradation, overcoming a common limitation of natural AMPs. To elucidate their mode of action, we employed molecular modelling to predict peptide-membrane interactions. These studies confirmed that the peptides compromise bacterial membrane integrity alongside membrane permeability assays. This finding was visually corroborated by scanning and transmission electron microscopy (SEM/TEM), which revealed irreversible structural damage and cell lysis. Furthermore, we evaluated kinetics of resistance development by exposing *E. coli* to serial passages of the peptides versus gentamicin. While the bacteria rapidly developed high-level resistance to gentamicin, no resistance was observed against our peptide candidates. These results show that the designed AMPs are potent and safe and offer a robust solution to resistance development. Their unique membrane-targeting mechanism, combined with a high safety profile and an inability to induce rapid resistance, positions them as promising candidates for future clinical development against multidrug-resistant infections.

S5. Innovation in Antimicrobial Stewardship and Optimized Clinical Strategies

The Impact of Intensive Care Unit Stewardship on the Appropriateness of Meropenem Prescription at a Tertiary Hospital: A Quasi-Experimental Study

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Background: Carbapenem-resistant Enterobacteriaceae (CRE) prevalence has shown increasing prevelance in Saudi Arabia, which poses a significant health concern. The antimicrobial stewardship program (ASP) plays an important role in reducing resistance by ensuring appropriate use of antibiotics.

Method: This is a retrospective data collection study performed over 3 years from 2020 to 2022, at a tertiary hospital which included adult patients who were hospitalized in the ICU and received Meropenem. The primary objective was to evaluate the appropriateness of meropenem prescription in the ICU pre-ASP and post-ASP implementation. The secondary objectives were assessing the reason for inappropriateness and the implemented action in response to inappropriate meropenem prescription.

Result: In total, 113 patients were included. The pre-ASP group included 34 patients; 41 patients were in the first year post-ASP intervention and 38 patients were in the second year post-ASP implementation. There was a significant increase in the number of patients who received appropriate prescriptions in the post-ASP intervention group 2021 and 2022 compared to pre-ASP implementation (26.8%, 53.7% vs 23.5%, p-value 0.002). The most observed reason for inappropriateness in the post-ASP group compared to the pre-ASP group varied (84.8% vs 76.9%, p value 0.53). Discontinuation of treatment in the post-ASP group was higher compared to the pre-ASP group (60% vs. 30.8%, p-value 0.036) meropenem use.

Conclusion: Approximately more than 70% of meropenem prescriptions were inappropriate, and the implementation of ASP shows a significant impact in promoting appropriate meropenem prescription patterns and shorter treatment durations in the ICU.

An Assessment of Factors Impacting Choice of Antibiotics in the Management of Neonatal Infections in a Neonatal Intensive Care Unit in a Resource-Limited Setting – a Pilot Project in Quality Improvement

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Introduction: Victoria Jubilee Hospital (VJH) has the largest NICU in Jamaica. Neonatal infections is a leading cause of morbidity and mortality. Quality improvement involves evaluation of antibiotic choices so as to enhance antibiotic stewardship.

Methods: Over three weeks, demographic and clinico-pathological data were reviewed along with indications for escalation and de-escalation of regimens. With no established antibiograms available, a line listing of confirmed infections was used as a proxy of the epidemiological profile of common infections.

Results:
21 neonates aged 0-20 days were reviewed. Gestational ages were 28 - 40 weeks, with 1/3 being premature. Birthweight ranged from 790-3610 gm. 17/21 (81%) received respiratory support, with one death during the period.

Major clinic-pathological conditions included:
Pneumonia (congenital & ventilator-associated, VAP)Necrotizing enterocolitisPersistent pulmonary hypertensionMeconium aspiration syndrome
20/21 neonates were commenced on empiric first line antibiotics – Amoxicillin /Amoxi-clavulanic Acid and Gentamicin. One infant was commenced on 2nd tier regimen (Piperacillin/Tazobactam & Amikacin; perinatal history of maternal chorioamnionitis), and was escalated to 3rd tier regimen by day 7 (Vancomycin and Meropenem) due to worsening clinical status.
7/21 infants were escalated to 2nd tier by day 7 (due to leukocytosis and worsening respiratory status; 6/7 had no positive cultures). One neonate grew Coagulase negative Staphylococcus and was treated for VAP. Although multi-drug resistant, with an in-vivo response, course was extended to 10 days' duration. There was no evidence of de-escalation of antimicrobials during the study period.

Conclusion: Antibiotic selection was primarily guided by overall epidemiological profile and clinical parameters rather microbiological results. Antibiotic de-escalation was not practiced. Stewardship strategies proposed include:

Phase A:
Increased availability of blood culture media and support from an in-house microbiology laboratoryProvision of adjunct biochemical studies including CRP & I/T ratio

Phase B:
Development of de-escalation protocols

Current State of Antimicrobial Stewardship in the Hungarian Human and Veterinary Health Sectors

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Introduction: Antimicrobial resistance (AMR) is among the top three health threats worldwide. It requires an effective response by implementing proper antimicrobial stewardship (AMS) measures. In Hungary, AMR is also a considerable problem, with overuse and misuse of antimicrobials. Despite still being one of the highest in Europe, Hungarian veterinary antibiotic consumption has significantly improved over the past decade. In contrast, however, the quantity of antibiotics used in human health care is relatively low, measured as DDD per 1000 inhabitants per day, but utilization and resistance patterns show worsening tendencies. In this study, we wanted to explore the quality of the current Hungarian AMS systems in human and animal health care and the barriers and facilitators of their operations.

Methods: We conducted a qualitative study using in-depth interviews with twelve Hungarian stakeholders representing the human, animal, and environmental sectors at ministerial, regulatory authority, and hospital/university levels. Semi-structured interviews were transcribed and analyzed using NVivo 15.

Results and discussion: Key informants revealed that the Hungarian veterinary antibiotic policy is greatly driven by the legal obligations of the EU Common Agricultural Policy, with its strong financial protection system focusing on food-producing animals. All sectorial interviewees emphasized that their national veterinary heath sector is aware of the problem of AMR and actively take all measures to achieve their goals. The identified facilitators were good intrasectorial communication and cooperation, ambitious leadership, efficient law-making, high-level education of vets, and the existence of antibiotic reduction strategies. In human health care, almost only pull-back factors were reported by the participants about the weak sectorial antibiotic policy, such as institutional (no dedicated leadership at any levels, no institutional framework, and lack of proper data collection and monitoring), juridical (no implemented national action plan, no legal background,and lack of funding), and personal factors (clinicians' resistance to change and the general population's low health literacy).

Does the Problem Begin at the Beginning? Assessment of Antimicrobial Resistance-Related Knowledge, Attitudes, and Practices Among Health Science Students: A Questionnaire-Based, Single-Centre, Cross-Sectional Study

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Introduction: Perspectives and competencies developed during undergraduate health education critically influence antimicrobial resistance (AMR)-associated attitudes and future clinical decision-making in healthcare staff, particularly with regard to fostering patient safety and rational antimicrobial utilization. The aim of our study was to assess nursing and allied health science students' knowledge, attitudes, and practices related to AMR.

Methods: A quantitative, single-centre cross-sectional study was performed with purposive sampling between 01/09/2021 and 20/09/2025. Data collection was carried out using an 83-item, self-administered online questionnaire consisting of the following domains: *i)* socio-demographic characteristics, *ii)* AMR-related knowledge, *iii)* attitudes towards AMR, *iv)* recognition of AMR-related terms, and *v)* practices related to antimicrobial consumption. Statistical analyses (descriptive statistics, Welch's t-tests, binary logistic regression, and a 95% confidence interval [95%CI]) were performed using IBM SPSS 28.0. The study followed the Checklist for Reporting Results of Internet E-Surveys (CHERRIES) guidelines.

Results: Among participants (*N*=312), 85.6% and 46.2% identified their formal health science training and the Internet as their main sources of AMR-related information, respectively. Participants enrolled in study programmes (e.g., nursing, dental hygiene) with stronger clinical and pharmacological components were more likely to possess adequate knowledge (OR=2.36; 95%CI: 1.49–3.71; *p*0.001), hold positive attitudes (OR=2.19; 95%CI: 1.38–3.46; p0.001), and recognize more AMR-related concepts (OR=2.95; 95%CI: 1.49–5.83; *p*=0.002). Overall, 51.3% demonstrated adequate knowledge, 59.3% had appropriate attitudes, while 15.4% correctly recognized a sufficient number of AMR-related concepts. In the past 12 months, 24.4% had used antibiotics (sources: medical prescription: 81.4%; leftover antibiotics: 6.7%; antibiotics from friends or family members: 7.4%), most commonly taking them to treat a throat ache (34.3%), fever (22.1%), or cough (18.9%).

Conclusions: Strengthening knowledge and attitudes toward AMR during undergraduate training is key to fostering evidence-based clinical decision-making in future practice. Continuous evaluation and enhancement of AMR-specific curricula should be considered ascore components of comprehensive AMR mitigation strategies.

Abstracts — Posters

Poster Session A

A01. Shorter Versus Longer Course of Antibiotics for Bloodstream Infections Caused by Carbapenemase-Producing *Klebsiella pneumoniae*: A Multicenter Study in Saudi Arabia

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Background: There is no consensus regarding the optimal duration of antibiotic therapy for carbapenemase-resistant *Klebsiella pneumoniae* bloodstream infections (CRKP-BSIs). We sought to compare survival in hospitalized patients with CRKP-BSIs who received shorter versus longer courses of antibiotics.

Methods: We conducted a propensity-score matched (PSM) retrospective multicenter study of adults who were diagnosed with CRKP-BSIs and received antibiotics for ≥ 72 hours. Our outcomes were 30-day mortality, 90-day mortality, and clinical cure. We compared patients treated with short (≤10 days) versus long (>10 days) antibiotic courses.

Results: Our PSM analysis included 97 patients. Of which, 32 patients received short-course antibiotics and 65 patients received long course antibiotics. We identified a significantly higher odds for clinical cure among those who received a course antibiotics (OR 1.25; 95% CI 1.02 – 1.53; p = 0.030). No difference was observed in 30-day and 90-day mortality between short and long antibiotic groups. Among patients with septic shock, receiving a prolonged course was associated with a significantly lower odds of 30-day mortality (OR 0.75; 95% CI 0.62 – 0.90; p = 0.002) and 90-day mortality (OR 0.75, 95% CI 0.65 – 0.86; p <0.001), and higher odds for clinical cure (OR 1.4, 95% CI 1.2 – 1.77; p <0.001)

Conclusion: For patients with CRKP-BSIs, 10 days of therapy may be sufficient; however, a longer duration of treatment may be warranted for septic shock patients.

A02. River Tributaries as Conduits of Antimicrobial Resistance: Genomics of *Escherichia coli* Draining into Laguna de Bay, The Philippines

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Laguna de Bay, the largest lake in the Philippines, is a critical multi-use water resource supporting fisheries, agriculture, recreation, and surrounding communities. It receives inflow from 21 river tributaries, draining densely populated areas where backyard farming, untreated wastewater inflow, and agricultural runoff are widespread. Unregulated antibiotic use in livestock and community settings may contribute to the emergence and spread of antimicrobial-resistant (AMR) bacteria across interconnected human, animal, and environmental compartments. Despite growing recognition of AMR as a One Health issue, data on environmental reservoirs of antimicrobial-resistance genes (ARGs) in Philippine freshwater systems remain limited.

In this study, Escherichia coli isolates were collected from river tributaries draining into Laguna de Bay and screened for ARGs using PCR. Isolates positive for extended-spectrum beta-lactamase genes, including blaSHV, bla TEM, and blaCTX-M-1, were selected for whole-genome sequencing using Oxford Nanopore long-read technology. Genome assembly and annotation were performed to characterize resistance gene profiles and their genomic context.

Whole-genome analysis revealed a diverse repertoire of ARGs, including clinically significant carbapenemase and oxacillinase genes not detected through PCR. The detection of blaNDM and blaOXA in environmental E. coli highlights the circulation of high-risk resistance determinants at the human–animal–environment interface. These findings indicate that river tributaries serve as important conduits for AMR dissemination into Laguna de Bay and underscore the value of genomics for One Health-oriented AMR surveillance in the Philippines.

A03. Hospital outbreak of VanA *Enterococcus faecium*: microbiological characterization and *in vitro* activity of oritavancin

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Introduction

Vancomycin-resistant *Enterococcus faecium* (VREfm) is an increasing challenge in hospital settings. Although oritavancin has shown in vitro activity against some VREfm isolates, its clinical role remains uncertain because clinical breakpoints and outcome data are limited.

Objectives

To describe the epidemiological and microbiological features of a hospital outbreak caused by vanA-positive *E. faecium.* To evaluate the in vitro activity of oritavancin.

Materials and methods

Bacterial identification was performed using MALDI-TOF mass spectrometry (bioMérieux VITEK® MS). The presence of vanA and related resistance determinants was assessed by next-generation sequencing (Oxford Nanopore Technologies).

Antimicrobial susceptibility was determined by broth microdilution using a commercial panel (ComASP® Oritavancin, Liofilchem).

Because EUCAST clinical breakpoints for oritavancin against *E. faecium*/VRE are not established, oritavancin MICs should be interpreted descriptively and compared with published MIC distributions rather than categorized as clinically susceptible or resistant.

Eight patients were identified as colonized with vanA-positive *E. faecium*. Cases clustered mainly in onco-hematology wards, suggesting nosocomial transmission in a high-risk population.

All isolates carried vanA and expressed high-level glycopeptide resistance, with:

- Vancomycin resistance (MIC ≥ 256 mg/L)
- Teicoplanin resistance (MIC ≥ 64 mg/L)

Oritavancin showed low in vitro MIC values against the outbreak isolates:

- MIC range: 0.25 mg/L
- Low MIC values were observed despite the presence of vanA. This finding is microbiologically relevant but should not be presented as evidence of clinical susceptibility in the absence of validated breakpoints and clinical outcomes.

Conclusions

Oritavancin demonstrated low in vitro MICs against the vanA-positive *E. faecium* outbreak isolates; however, these results should be considered descriptive because validated clinical breakpoints for this organism-drug combination are lacking.

Isolates showed high-level glycopeptide resistance, but retained low oritavancin MICs. Further PK/PD, synergy and clinical-outcome studies of oritavancin in VREfms to establish oritavancin as a standard therapeutic alternative for invasive infection are needed.

A04. Evaluation of the Synergistic Activity and In Vitro Efficacy of Imipenem/Relebactam in Combination with Amikacin and Tigecycline Against Clinical *Burkholderia cepacia* Complex Isolates

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Objectives
The *Burkholderia cepacia* complex (BCC) comprises significant opportunistic pathogens frequently associated with healthcare-related infections and high levels of multidrug resistance. Due to extensive intrinsic and acquired resistance mechanisms, these infections pose a major therapeutic challenge. This study aimed to investigate the in vitro activity of imipenem/relebactam (IMI/REL) and to evaluate its synergistic effects when combined with amikacin (AMK) and tigecycline (TGC) against clinical BCC isolates.

Methods
A total of 20 non-epidemiologically related clinical BCC isolates were included. Minimum inhibitory concentrations (MICs) were determined by means of the broth microdilution method according to CLSI (2023) guidelines. Synergistic potential was assessed using time-kill assays following CLSI M26-A standards.

Results
IMI/REL showed 80% susceptibility against the tested BCC isolates. Based on MIC_{50} and MIC_{90} values, the activity profiles were as follows: IMI/REL (1/4 and 128/4 mg/L), TGC (2 and 4 mg/L), and AMK (32 and 128 mg/L). Time-kill analyses revealed that the IMI/REL–AMK combination produced the most pronounced synergistic effect, achieving ≥3 $\log_{10}$ reduction in bacterial counts relative to the initial inoculum within 24 hours. Sustained bactericidal activity was observed throughout the experiment. No antagonistic interactions were detected among the tested combinations.

Conclusion
Our findings indicate that IMI/REL possesses significant in vitro activity against clinical BCC isolates. The observed synergy and bactericidal activity underscore the IMI/REL–AMK combination as a promising therapeutic strategy for combating infections caused by this challenging, multidrug-resistant pathogen.

A05. Antimicrobial Resistance Profiles of *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Cattle Farms within a One Health Framework

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Antimicrobial resistance (AMR) is a major global threat to human, animal, and environmental health, particularly in livestock production systems with frequent antibiotic use. Food-producing animals can act as reservoirs for resistant bacteria, facilitating their spread through environmental pathways and the food chain. In this context, *Escherichia coli* and *Klebsiella pneumoniae* are widely used indicator organisms for monitoring antimicrobial resistance within the One Health framework.
This study aims to investigate the antimicrobial resistance profiles of *Escherichia coli* and *Klebsiella pneumoniae* isolated from cattle farms and to provide preliminary data on resistance patterns in animal and environmental samples. Sampling was conducted in four cattle farms, and 120 samples were collected, including fecal, milk, water, feed, and environmental surface samples. Following collection, samples were pre-enriched in buffered peptone water and subsequently cultured on Endo agar and chromogenic UTI agar for the selective isolation of *Enterobacteriaceae*. Presumptive colonies were selected based on colony morphology and subcultured to obtain pure isolates, which were identified using conventional biochemical methods.
Antimicrobial susceptibility testing was performed using the disk diffusion method in accordance with CLSI and EUCAST guidelines. The antimicrobial panel included β-lactams (ampicillin, cefotaxime, ceftazidime), fluoroquinolones (ciprofloxacin), aminoglycosides (gentamicin), tetracyclines, and sulfonamides, representing antibiotics commonly used in veterinary practice. Extended-spectrum β-lactamase (ESBL) production was evaluated using phenotypic confirmatory tests.
To date, 70 *Enterobacteriaceae* isolates have been obtained (62 *Escherichia coli* and 8 *Klebsiella pneumoniae*), and sampling is ongoing with an expected final collection of approximately 90 isolates. Antimicrobial susceptibility testing and ESBL screening are currently in progress, and the final analysis will focus on multidrug resistance patterns and the prevalence of ESBL-producing strains. The findings of this study are expected to provide insight into antimicrobial resistance trends in cattle farms and support the One Health approach by highlighting links between antibiotic use in livestock, environmental contamination, and public health risks.

A06. Phenotypic Profiling of Biofilm Formation and Antibiotic Susceptibility in Poultry-Derived *Listeria monocytogenes* Isolates

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Listeria monocytogenes is a critical foodborne pathogen, with poultry products serving as a potential reservoir. Its ability to form biofilms may aid in its persistence on processing equipment and food-contact surfaces, while antimicrobial resistance complicates efforts to control and treat infections. This study analyzed 93 *L. monocytogenes* isolates from poultry to assess their biofilm-forming capacity and antibiotic resistance. Biofilms were tested on polystyrene micro-titer plates at 12 °C and 30 °C in a nutrient-rich medium (Brain Heart Infusion, BHI). Susceptibility to eight antibiotics relevant to clinical and food safety was tested by disk diffusion and evaluated using EUCAST breakpoints, where available. Most isolates produced detectable, typically weak biofilms at both temperatures, with some shifting to moderate biofilm formation after extended incubation at 12 °C; no strong biofilm producers were observed. This underscores the significant role of incubation time and temperature in surface colonization. Overall, the isolates remained mostly susceptible to ampicillin, penicillin G, vancomycin, tetracycline, and chloramphenicol, whereas some subpopulations exhibited resistance or low susceptibility to trimethoprim–sulfamethoxazole (TMP-SMX) and, notably, erythromycin and streptomycin. No consistent link was found between biofilm formation and antibiotic susceptibility, suggesting these traits are largely independent among these isolates. These results demonstrate that poultry-derived *L. monocytogenes* can form weak to moderate biofilms under the tested monoculture conditions while generally remaining susceptible to first-line antibiotics. Nevertheless, the emergence of resistance to macrolides and aminoglycosides, together with the potential for increased colonization in complex multispecies biofilms in real food processing environments, underscores the need for ongoing, integrated surveillance across animal food systems.

A07. Addressing Antimicrobial Resistance: The Potential Role of Homeopathy

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AMR poses a significant threat to global health, with more than 700,000 yearly deaths worldwide. There is an urgent need to reduce antibiotic use, and Primary care and agriculture are major targets for antimicrobial stewardship. Global, regional, and national strategies were developed in the last decade to reduce AMR. However, little progress has been made despite all significant efforts to reduce antibiotic use and AMR. Given the magnitude of the problem, the insufficient strategies to reduce antimicrobial use and the urgency and impact of AMR, there is a need for novel strategies. Traditional, Complementary and Integrative Medicine (TCIM) may provide strategies and solutions that contribute to reducing (inappropriate) antibiotic use, specifically as part of a delayed prescription strategy or as an alternative/ add-on prevention or treatment strategy in both human and veterinary medicine. Homeopathy is one of the most popular TCIM strategies widely used worldwide. The use of homeopathy in the prevention and treatment of infections is based on increasing scientific studies and evidence. To illustrate the substantial role homeopathy could play in helping to reduce the problem of AMR an overview of high-quality studies, including Systematic Reviews and Meta-analysis, Double-Blind Placebo-Controlled Randomised Clinical Trials (RCT) and Real-World Evidence (RWE) studies will be presented. In addition, HRI's research programme on homeopathy and antimicrobial resistance (ENHANCE), which, to date, includes three systematic reviews and meta-analysis (Otitis Media, Tonsilitis and Sinusitis) and studies on the determination and validation of Core Outcome Sets (COS) for future primary care infection research (Acute Otitis Media, Tonsillitis and Sinusitis) will be introduced. This initiative, through a combination of high-quality research, interdisciplinary collaboration, and standardised methodologies, will strengthen the evidence assessing homeopathic treatment for primary care infections, ensuring safe and effective treatment alternatives for patients and thus contributing to the long-term goal of reducing antibiotic dependence.

A08. Prevalence of Vancomycin Resistance Genes in Polish Hospital Wastewater

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Resistance to vancomycin in bacteria (VRB) is primarily mediated by *van* genes (VRGs), among which the *van*A and *van*B phenotypes have the greatest clinical significance. They can be transferred between microbial cells via mobile genetic elements, which determines the high epidemic potential of these strains, creating even more dangerous "superbugs". Hospital wastewater (HW) discharged to wastewater treatment plants (WWTPs) is the main anthropogenic source of VRB and VRGs in municipal wastewater, which are finally released into the environment. Therefore, the main goal of this study was to assess the concentration of VRGs in hospital wastewater collected from the whole territory of Poland.

In this study, HW samples were collected in winter and summer seasons from 64 hospitals located in various provinces of Poland. Environmental DNA wasextracted from HW using the FastDNA™ Spin Kit for Feces (MP Biomedicals). The concentration of VRGs was analysed by high-throughput long-read sequencing based on nanopore technology.

In both sampling seasons (winter and summer, 2024), qualitative and quantitative analyses of VRGs revealed considerable differences in gene counts between seasons and regions of HW sample collection. The abundance of VRGs ranged from 0 to 6.55×10^2 gene copies/Gb and was highest in HW collected in winter from central-eastern Poland. Among ARGs responsible for resistance to vancomycin, *van*A and *van*B genes presented most frequently and the most common hosts of VRGs were *Enterococcus faecium* and *Staphylococcus aureus*.

The results of this study prove that untreated HW is a hotspot for VRGs and VRB to enter the environment. This research can be used to clarify the requirements for hospitals regarding the disinfection of wastewater, as well as the requirements for microbiological quality of wastewater entering WWTPs.

A09. Isolation and Characterization of Bacterial Strains Resistant to Beta-lactam Antibiotics, Including Carbapenems, From the Polish Baltic Sea Coast

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In response to the growing threat posed by increasing microbial resistance to antimicrobial agents, the World Health Organization has developed the “One Health” concept, which recognizes the interconnectedness of human, animal, and environmental health. The BALTIC-AMR project aims to develop a rational, transnational strategy for countries surrounding the Baltic Sea, enabling a comprehensive analysis of and response to the growing phenomenon of resistance to antibacterial compounds.
The present study focuses on the isolation of bacterial strains that are resistant to β-lactam antibiotics and carbapenems from sewage samples (raw and treated) as well as from seawater collected in coastal areas of the Baltic Sea in Poland. Sampling was conducted every two weeks during 2024 and 2025. Strains were isolated using the membrane filtration method and cultured on CHROMagar™ ESBL and mSuperCARBA™ media. Biochemical identification of the isolates was performed using the VITEK 2 automated system (bioMérieux).
In total, 98 strains were analyzed, including 53 that were resistant to cefotaxime and 45 that were resistant to meropenem. The majority of isolates belonged to the genus *Pseudomonas* (23%), *Escherichia coli* (20%), and the *Enterobacter cloacae* complex (18%). The remaining isolates were identified as *Klebsiella pneumoniae* subsp. *pneumoniae*, *Citrobacter freundii*, *Citrobacter braakii*, *Stenotrophomonas maltophilia*, and several other species, including *Serratia fonticola*, *Hafnia alvei*, and *Aeromonas sobria*. In the next stage of the study, whole-genome sequencing of the bacterial isolates will be performed to further characterize their antimicrobial resistance profiles. Overall, these findings highlight the environmental dissemination of antimicrobial-resistant bacteria and underscore the importance of integrated surveillance within the One Health framework.
Project No. 2023/05/Y/NZ9/00169 funded by the National Science Centre in cooperation with the JPIAMR network - Joint Programming Initiative on Antimicrobial Resistance.

A10. Antimicrobial resistance of *Salmonella typhimurium* in Pig Production in Poland: Phenotypic and Genotypic Analysis

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Introduction:
Salmonella enterica serovar Typhimurium remains one of the most important zoonotic pathogens associated with pig production. Its ability to persist and disseminate multidrug resistance (MDR) poses a significant challenge within the One Health framework. This study aimed to characterize phenotypic and genotypic antimicrobial resistance among *S.* Typhimurium isolates obtained from pigs and their environment in Poland between 2014 and 2019.

Materials and Methods:
From a collection of approximately 580 *Salmonella* isolates of different serovars collected from pigs and related environments in Poland between 2014 and 2019, 36 *Salmonella* Typhimurium (including monophasic and atypical variants) isolates were selected for whole-genome sequencing and further analysis. Isolates originated from feces/manure, boot swabs, environmental dust, rectal swabs, and pork. Antimicrobial susceptibility was determined using the broth microdilution method with EUVSEC plates (TREK Diagnostic Systems), covering 14 antimicrobial agents. Minimum inhibitory concentration (MIC) values were interpreted according to EUCAST epidemiological cut-off values (ECOFFs). Whole-genome sequencing (WGS) was performed on the MiSeq platform (2×300 bp; Illumina). Sequence data were analyzed using Staramr tools implemented in Galaxy ARIES.

Results:
MIC above ECOFF values were observed for ampicillin (≥32 mg/L), sulfamethoxazole (≥512 mg/L), tetracycline (≥32 mg/L), trimethoprim (≥16 mg/L), and nalidixic acid (≥128 mg/L). Reduced susceptibility to ciprofloxacin correlated with mutations in *gyrA*. All isolates were susceptible to medically important meropenem and colistin. Two major clonal lineages predominated: ST19 (n = 12) and ST34 (n = 24). ST19 isolates most frequently harbored $bla_{\text{CARB-2}}$, *floR*, *tet*(G), *sul1*, and *aadA2*, together with the *gyrA* (D87N) mutation. ST34 isolates showed more diverse MDR profiles, including $bla_{\text{TEM-1B}}$, *sul2*, *tet*(A/B), *aadA1*, *aph*, and sporadically *qnrS1*.

Conclusions:
The study demonstrates the circulation of MDR *Salmonella enterica* serovar Typhimurium ST19 and ST34 clones in Polish pig production between 2014 and 2019, highlighting the value of integrated AMR surveillance within the One Health approach.

A11. Steady-State Concentrations of Clarithromycin Under Different Routes of Administration in Pneumonia: Risk Factors and Clinical Outcomes

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Appropriate antibiotic dosage is crucial for improving outcomes in critically ill patients facing pneumonia. Our research aimed to evaluate the development of the steady-state concentration (Css) of clarithromycin (CLAR) in this specific patient population with different routes of administration, to identify the influencing factors, and to examine the relationship between clinical outcomes and Css. This study was conducted as a single-center prospective observational study in the ICU of a pulmonology department between February 2025 and December 2025. The study target group included all adult patients (n=72) treated primarily with empirical CLAR (2x500mg/day) due to pneumonia. Blood sample collection was performed on day 3 of the CLAR administration, when five samples were taken as follows: at steady-state before drug administration and 1, 2, and 3 hours after intravenous (IV), oral (PO), and nasogastric (NG) administration, and one sample for albumin level. Determination of CLAR serum concentrations was determined by LC-MS/MS using a CLAR European Pharmacopeia Reference Standard by Merck. CLAR serum levels followed the expected pharmacokinetics for all three routes of administration. The CLAR Css was 3-fold higher for PO (n=30) than for IV (n=27) administration and 2.7-fold higher for NG (n=15) administration. Severe reduction in CrCl (< 59ml/min) increased serum levels for all administration routes. CLAR serum levels increased proportionally with increasing albumin serum levels and with the increasing CCI (Charles Comorbidity Index). No difference in length of stay (LOS) and survival was found between IV and PO administration (81 and 83%); however, the probability of survival was significantly lower (40%) in the case of NG administration. PO administration presented the highest CLAR Css levels. CrCl, albumin levels, and CCI were found to be influencing factors of CLAR Css. Although PO and IV administration resulted in similar clinical outcomes, the lower survival rate associated with higher Css in the case of NG administration requires further investigation.

A12. Identification of an SCCmec-mecC Hybrid in Methicillin-Resistant *Mammaliicoccus sciuri* from Australian Wildlife

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Background:
Mammaliicoccus sciuri, previously known as *Staphylococcus sciuri*, is a commensal bacterium widely distributed in animals and an opportunistic pathogen associated with infections in animals and humans. *M. sciuri* may act as a reservoir of antimicrobial resistance determinants, including the staphylococcal cassette chromosome *mec* (SCC*mec*) element, which confers methicillin resistance in methicillin-resistant *Staphyloccoccus aureus* (MRSA) via the *mecA* or *mecC* gene. In this study, we characterized methicillin-resistant *M. sciuri* (MRMS) isolated from Australian wildlife.

Method:
Skin and nasal swabs were collected from 180 wild animals upon admission to a wildlife hospital in Western Australia, and from 97 animals after seven days of hospitalization. ChromID MRSA selective agar was used for the isolation of MRMS. Whole genome sequencing was performed using the Illumina NovaSeq 6000 platform and the Oxford Nanopore MinION. The PubMLST website was used to determine the sequence type (ST). SCC*mec* elements were annotated and analyzed using the Geneious Prime software.

Results:
Thirty-one MRMSs were isolated from 4.4% (8/180) of the animals upon admission and 23.7% of animals (23/97) after hospitalization. Three STs were identified—ST81 (n=2), ST321 (n=16) and ST337 (n=13). All MRMS isolates harboured an SCC*mec* element. The ST81 and ST337 MRMSs harboured an *mecA*-positive SCC*mec* III(3A); an SCCmec was reported in hospital-associated MRSA clones. In contrast, the ST321 MRMS harboured an SCCmec-*mecC* hybrid element which contained the *mecA*, *mecC* and *blaZ* resistance genes. The SCC*mec*-*mecC* hybrid consisted of the SCC*mec* III(3A) element fused to a partial SCC*mec* XI element.

Conclusion:
Methicillin-resistant *M. sciuri* isolated from Australian wildlife carries *mecA* or *mecC* on an SCC*mec* element. This study provides the first evidence of an SCC*mec*-*mecC* hybrid in *M. sciuri* in Australia.

A13. Metagenomic Insights Into Circulation and Maintenance of Antibiotic Resistome in Intensive Dairy Farm Environments

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Antibiotic resistance is an urgent global public health threat, with livestock recognized as a potential reservoir for antibiotic resistance genes (ARGs). Despite the extensive antibiotic use in dairy farms, little is known about ARG dynamics in these environments. This study was conducted on five intensive dairy farms in Greece to explore the presence of ARGs across the production cycle and potential exposure sources. Feces from lactating cows, pre-weaned and weaned calves, bulk-tank milk and colostrum, and environmental samples, including dust from feeding alleys and beams and air samples taken during routine farm activities, were collected. Stored solid and liquid manure and material from corresponding biogas treatment plants were also sampled. Shotgun metagenomic short-read sequencing was conducted on extracted DNAs to identify the antibiotic resistomes (ARG loads) and characterize microbiomes. Sequencing reads were aligned to reference ARGs (CARD, MEGARes, and ResFinder) and microbiome (Kraken) databases. ARGs to aminoglycosides, macrolide–lincosamide–streptogramin, tetracycline, sulfonamide–trimethoprim and β-lactams were abundant across samples. Pre-weaned calves carried high ARG loads, contrary to colostrum, reflecting increased antibiotic selective pressure in this age. Intriguingly, beam-dust samples harbored high load and diverse ARGs, including *aac(6), aadA, aph(6), ant(6), aac(3), erm(A/B/F/X/C/35*), *mef(A), lnu(C/G), tet(A/H/K/L/M/Q/X), dfrA/D*, *sul1/2*, and *mcr* and the ESBLs $bla_{TEM,}$ $bla_{OXA,}$ and $bla_{CTX-M.}$ Unexpectedly, bla_{IMP} conferring resistance to carbapenems was detected in both animal and environmental samples, despite these antibiotics are not authorized for use in livestock. ARG maintenance in dust, along with lower but similar loads in air, poses a potential exposure risk for humans. Finally, biogas treatment samples, regardless of their differing microbiomes, were not free of ARGs and thereby could create, as fertilizers, an important channel for ARG transmission to humans and animals. In conclusion, avoidance of antibiotic misuse, application of strict biosafety measures and establishment of systematic surveillance are imperative for intensive dairy farming.

A14. Dogs and Cats as a Potential Reservoir of Resistant *Escherichia coli*

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Aim: Companion animals such as dogs and cats are increasingly recognized as potential reservoirs of antimicrobial resistance (AMR) determinants with relevance to both veterinary and human medicine. This study aimed to characterize antimicrobial resistance profiles of *Escherichia coli* isolated from dogs and cats in 2024 using a combined phenotypic and genomic approach.

Materials and methods: A total of 115 fecal samples collected from 63 dogs and 52 cats from animal shelters were screened for the presence of cephalosporin-resistant *Escherichia coli*. A total of 65 isolates were obtained and tested for antimicrobial resistance using the broth microdilution method with EUVSEC3 and EUVSEC2 plates (TREK Diagnostic Systems). Results were interpreted according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) ECOFFs. Whole-genome sequencing (WGS, 2×300 bp, NextSeq platform, Illumina) was performed for selected isolates to identify AMR genes and plasmid replicons.

Results: Phenotypic testing revealed a high level of resistance to critically important antimicrobials. Resistance to third-generation cephalosporins was observed in nearly all isolates (100% for cefotaxime and 98.5% for ceftazidime), while resistance to ampicillin reached 100%. Similar to ampicillin, ciprofloxacin resistance was observed in all isolates. High levels of resistance were also detected for nalidixic acid (92.3%), tetracycline (98.5%), chloramphenicol (95.4%), and gentamicin (90.8%). In contrast, all isolates remained fully susceptible to critically important last-resort antimicrobials, including meropenem, imipenem, colistin, and amikacin. Resistance to tigecycline, trimethoprim and sulfamethoxazole was detected at lower frequencies.
WGS revealed a predominance of *E. coli* ST457, suggesting clonal dissemination among companion animals. Most isolates carried ESBL genes, primarily $bla_{\text{CTX-M-32}}$, with single isolates harbouring $bla_{\text{CTX-M-15}}$ or $bla_{\text{CTX-M-55}}$. The IncF replicons (IncFIA/IncFIB) were common.

Conclusions: Dogs and cats may serve as an important reservoir of multidrug-resistant and ESBL-producing *E. coli*, highlighting the need for AMR surveillance in companion animals within a One Health framework.

A15. T3SS Virulence Factors and Antimicrobial Resistance of *Pseudomonas aeruginosa* Isolated From Medical Devices of Peruvian Inpatients in Critical Care Units

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Pseudomonas aeruginosa is an opportunistic Gram-negative bacteria identified as the main cause of serious nosocomial infections in Peru, especially in vulnerable patients, such as those hospitalized in critical care units (CCUs). Often, these infections are difficult to treat due to high antimicrobial resistance, enhanced virulence causing greater damage to the host, a tendency towards chronicity, and increases the risk of mortality. Thus, surveillance of this species in the aforementioned wards is encouraged.
Between July and September 2025, we collected the following medical devices, vascular catheters (VCs), urinary catheters (UCs), and endotracheal tubes (ETTs), from adult patients hospitalized in the CCU of a level-III reference hospital. Forty-two *Pseudomonas aeruginosa* isolates from different devices were recovered from MacConkey Agar and confirmed by PCR. Antimicrobial susceptibility was performed by disk diffusion using CLSI 2025 guidelines. Type 3 Secretion system virulence factor genes (T3SS) were detected by PCR, as well as carbapenemase genes bla_{VIM}, bla_{IMP}, bla_{NDM} and bla_{KPC}. Out of 42 strains of *P. aeruginosa* from medical devices, 76% (32/42) were carbapenem-resistant; bla_{IMP} was identified in 12/32 isolates and bla_{VIM} in 5/32, the latter being VIM/IMP co-producers. The bla_{KPC} gene was found in 6/32 isolates and none carried bla_{NDM}. Regarding T3SS, we found the presence of *exoU* (15/42) and *exoS* (30/42), with three isolates being *exoU*+/*exoS*+. Most isolates also carried *exoY* (42/42) and *exoT* (39/42).
These results showed high carbapenem resistance in *P. aeruginosa* isolated from medical devices at a III-level Peruvian hospital and shed light on its T3SS virulence factors.Similar molecular epidemiology studies should be replicated.

A16. Detection of Antibiotic Resistance and Virulence Genes in *Salmonella* Strains Isolated From Refrigerated Raw Retail Chicken in Two Regions of Chile

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Salmonella enterica is a major cause of foodborne illness, resulting in over 90 million cases and 150,000 deaths annually worldwide. Found in water and foods like meat and milk, it generally causes gastroenteritis, requiring antibiotic treatment only in severe cases. However, increasing antibiotic resistance in this species presents a significant public health challenge globally. Objective: To genetically characterize *Salmonella* strains isolated from raw retail chicken in the Ñuble and Maule regions of Chile. Methodology: A total of 225 raw chicken meat samples (175 from Maule and 50 from Ñuble) were analyzed under the Salmonella surveillance program using sampling criteria n=5, c=0. Isolates were identified by the VIDAS system, confirmed by MALDI-TOF, and sequenced on the MiSeq. Classic MLST, core genome MLST, and *Salmonella* In Silico Typing Resource (SISTR v1.1.3) were performed. Antibiotic resistance genes (ARG) were detected using AMRFinderPlus and virulence genes (VG) by AMRfinderPlus templates (MBioSEQ Rido Typer v11.1). Nine Salmonella strains were isolated from independent samples, of which eight were *S.* Infantis ST32 (CC31; 6,7,14: r:1,5) and one was *S.* Bredeney ST897 (CC33; 1,4,12,27: l, v:1,7). Three clusters of closely related strains were observed with 0-2 allele differences, and three unrelated strains were identified. IncFIB plasmids were common in *S.* Infantis and Col_rep_cluster in *S.* Bredeney. *S.* Infantis strains presented the ARGs *aac(3)-IVa, aph(3')-Ia, aph(4)-Ia, aadA1, mdsA/mdsB, fosA3, floR, qacEdelta1, gyrA_D87Y, sul1, tet(A)* and *dfrA14. bla*$_{CTX\text{-}M\text{-}65}$(ESBL) was detected only in IncFIB plasmids . In *S.* Bredeney, the ARGs detected *were aac(3)-IId, aph(3')-Ia, aadA2/aph(3")-Ib/aph(6)-Id, blaTEM-1, mdsA/mdsB, lnu(G), floR, qnrB19, sul2, tet(A)/tet(B),* and *dfrA12*. A total 147 VG were detected, highlighting exotoxin, adherence, and effector delivery systems. Conclusion: The analyzed *Salmonella* strains exhibited various ARG and VG, underscoring the need for continuous genomic surveillance to mitigate public health risks in Chile.

A17. Seasonal Dynamics of the Airborne Resistome and Mobilome across Diverse Environments in the Belgrade Metropolitan Area

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Introduction
The role of the environment in the dissemination of antimicrobial resistance is increasingly recognized within the One Health framework. The airborne transmission route of antibiotic resistance genes (ARGs) is particularly significant since ARG-carrying bioaerosols could travel long distances and remain in the atmosphere for a long period of time. In addition, mobile genetic elements (MGEs) can promote the spread of ARGs in the environment.

Objective
This study aimed to characterize the seasonal dynamics of the airborne resistome and mobilome across diverse environments in the Belgrade metropolitan area, Serbia.

Methods
Outdoor air samples were collected at several urban, suburban, and rural locations across four seasons. Airborne DNA was subjected to shotgun metagenomic sequencing (Illumina NovaSeq X Plus), followed by bioinformatic analysis. Resistance genes were annotated using MEGARes, while integrons, insertion sequences (ISs), and plasmids were identified using Integrall, ISfinder, and PLSDB, respectively.

Findings
Shotgun metagenomic analysis revealed pronounced seasonal variation in the composition and abundance of the airborne resistome across all sampled environments. ARGs dominated the resistome across all seasons, followed by metal resistance genes (MRGs) and multi-compound resistance genes (MCRGs). Resistome abundance and gene richness increased progressively from spring to winter, accompanied by a higher number of season-specific resistance genes during autumn and winter. In addition, the airborne mobilome was dominated by integron- and plasmid-associated sequences, while ISs accounted for a smaller proportion of the total mobilome. Total MGE abundance exhibited a modest seasonal trend, with winter tending to show higher levels than other seasons. MGE diversity and the number of season-specific elements increased toward winter, driven mainly by plasmid-derived sequences.

Conclusion
Our results demonstrate that air represents a dynamic environmental reservoir and potential dissemination pathway for antimicrobial resistance across the Belgrade metropolitan area, emphasizing the importance of incorporating airborne environments into One Health surveillance strategies.

A18. Study of Antimicrobial Susceptibility of *Enterococcus spp.* Isolated From Wild Mammals From the Autonomous Community of Aragón (Spain)

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Enterococcus spp are present in the gastrointestinal tract of animals, humans and in the environment. The most important are *E. faecalis* and *E. faecium*, but other enterococci can produce nosocomial infections. Enterococci have intrinsic and acquire mechanisms of antimicrobial resistance easily. Vancomycin is the last line of defense against these infections. The resistance acquired through the VanA and VanB2 genes is easily transmitted
The aim of this study is to determine if enterococci isolated from wild mammals of the Aragón Community (Spain), exhibit resistance to important antibiotics and carry genes of vancomycin resistance.
Between 2022 and 2024, 99 rectal samples were collected from recently dead mammals at the Wildlife Recovery Center-La Alfranca. After isolation on Slanetz-Barley agar, they were identified in MALDI-TOF-MS. The Kirby-Bauer test was applied to detect susceptibility to antimicrobials. The *vanA* and *vanB* genes were searched by PCR, with specific primers.
The mammal species were 33 badger, 23 beech marten, 11 American mink, 9 hedgehog, 6 common otter, 4 wild cat, 4 red fox, 3 common genet, 3 bat, 1 wolf, 1 guinea pig and 1 ferret.
It was isolated 126 enterococci: 63 *E. faecalis*, 31 *E. faecium*, 14 *E. hirae*, 11 *E. mundtii*, 2 *E. avium*, 2 *E. gllinarum*, 1 *E. durans*, 1 *E. villorum* 1 *E. casseliflavus*
The intermediate halo for the erythromycin, ciprofloxacin, and vancomycin was observed in 82, 56, and 29 enterococci, respectively. Thirty-three enterococci were resistant to tetracycline. None enterococci was resistant to clhoramphenicol, linezolid and teicoplanin. One *E. faecalis* was vancomicyn resistant. Ninenty strains were selecte to search for VanA and VanB2 genes, and were negative.
Conclusion: Enterococci isolated from 99 wild mammals in Aragón (Spain) showed high susceptibility to the antibiotics tested and did not carry vanA or vanB2 genes.

A19. Environmental Reservoirs of Carbapenem-Resistant *Escherichia coli* ST131 and ST69 in Zambia: A One Health Genomic Study

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Introduction: Carbapenem-resistant *Escherichia coli* (CREC) poses a critical public health threat globally. High-risk clones ST131 and ST69 drive the pandemic, yet their epidemiology in sub-Saharan Africa remains poorly characterized. We investigated the genomic features and epidemiological patterns of *E. coli* from human, animal, and environmental sources in Zambia using a comprehensive One Health approach.

Methods: Whole-genome sequencing was performed on 97 *E. coli* isolates (57 clinical, 40 environmental) collected from Lusaka, Zambia. Antimicrobial susceptibility testing, MLST typing, phylogroup assignment, and identification of AMR genes, plasmid replicons, and virulence factors were conducted. Statistical analyses included chi-square tests, Fisher's exact test, and principal component analysis to identify significant associations.

Results: Overall, 82.5% of isolates were multidrug-resistant. Carbapenem resistance was detected in 16.5% of isolates, significantly higher in environmental (25.0%) than clinical (10.5%) sources. The carbapenemase *bla*NDM-5 was exclusively found in environmental isolates (7.2%). ST131 dominated clinical isolates (41.2%), while ST69 was predominant environmentally (45.0%). Phylogroup B2 was most common clinically (50.9%), whereas phylogroup D was more prevalent environmentally (32.5%). Significant associations existed between specific STs and AMR genes (*bla*TEM-1, *bla*OXA-1 with ST131; $p<0.01$).

Conclusions: Environmental reservoirs in Zambia harbor a significant and concerning CREC burden, particularly ST69 and *bla*NDM-5-producing isolates. The distinct distribution of ST131 and ST69 between clinical and environmental settings suggests separate epidemiological pathways and transmission routes. These findings highlight the environment as a critical hub for CREC evolution and transmission, emphasizing the urgent need for integrated One Health surveillance and infection control strategies in the region.

A20. Pharmacists' Observations and Attitudes Towards Medication Supply and Community Self-Medication Intentions During The COVID-19 Pandemic: A Qualitative, Descriptive Study

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Introduction: During the early stages of the COVID-19 pandemic—when many health services were inaccessible—the role of community pharmacists became particularly prominent, playing key roles in pharmaceutical care and patient education. This study aimed to explore the perceptions and attitudes of community pharmacy staff regarding the conditions of medication supply and patterns of self-medication, with a focus on antimicrobials, during the first year of the COVID-19 pandemic.
Methods: A qualitative, descriptive study was conducted using semi-structured, focus-group interviews with community pharmacy staff in the Southern Great Plain of Hungary. Data collection was carried out between 15/05/2020 and 15/02/2021, using a non-probability sampling approach. Interviews were conducted online via the Zoom™ videoconference platform, transcribed manually, and analysed via thematic analysis through an inductive approach. A total of *N*=16 pharmacists participated across four focus groups (3–5 participants per group). Recruitment continued until saturation was achieved, i.e. when no new relevant information emerged. The study followed the Consolidated criteria for Reporting Qualitative Research (COREQ) guidelines.
Results: Of the participants, 11/16 were women, and their median age was 35 years (range: 26–57); 6/16 had board certifications in pharmacy management. The following major themes were identified: *(i)* the role of the National eHealth Infrastructure (EESzT) and e-prescriptions during the pandemic; *(ii)* the emergence and monitoring of COVID-19 "miracle cures" (e.g., hydroxychloroquine, ivermectin, azelastine) and medicines "to be avoided" (e.g., ibuprofen, ACE inhibitors); and *(iii)* the legitimization of inappropriate antibiotic use through "defensive medicine".
Conclusions: Rapid dissemination of health-related information—regardless of its basis in evidence—shaped public interest in the perceived efficacy and availability of certain medicines; the trend of "information noise" generated by the media was clearly reflected in community pharmacies. Patterns of antibiotic self-medication have also shifted, as many patients obtained antimicrobials through physician prescriptions issued for "prophylactic" purposes during the pandemic.

A21. Silent Invaders: When Neonatal Devices Become Gateways to Antibiotic Resistance

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Background and Aims
In the delicate realm of neonatal care, peripheral venous catheters (PVCs) are indispensable but also serve as potential gateways for catheter-associated infections (CAIs). These infections, often caused by *Staphylococcus aureus*, including methicillin-resistant strains (MRSA), contribute significantly to the growing challenge of bacterial resistance in hospital settings. This study aimed to assess catheter colonization, characterize resistance profiles—especially methicillin resistance—and identify factors linked to infection risk. Particular attention was given to *Staphylococcus* spp. and their resistance mechanisms.

Methods
We collected 403 catheter tip samples from 325 neonates hospitalized in NICUs. Bacterial colonization was evaluated using the quantitative Cleri–Brun–Buisson method. Emphasis was placed on *S. aureus*. Methicillin resistance was confirmed through PCR detection of the *mecA* gene. We conducted a genetic diversity analysis of *mecA* sequences to explore clonal variation. Additionally, the D-test was used to detect inducible clindamycin resistance, offering insight into phenotypic resistance expression.

Results
Colonization by MRSA and methicillin-resistant CoNS was identified in a subset of samples. Prolonged catheter dwell time and inconsistent aseptic technique were associated with higher colonization rates. The *mecA* gene was detected in several isolates, displaying notable genetic diversity indicative of multiple circulating clones. The D-test revealed inducible clindamycin resistance in various strains, underscoring the relevance of phenotypic testing.

Conclusion
Our findings highlight the need to reinforce infection prevention protocols and closely monitor bacterial resistance in NICUs. The diversity of *mecA*-positive strains and the detection of inducible resistance patterns underscore the importance of combining molecular and phenotypic methods. Improved catheter management and targeted surveillance can significantly reduce CAIs and enhance the protection of vulnerable neonatal patients.

A22. Antibiotic Treatment in Humans And Companion Animals – Is a Pet Like a Human?

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Aim and scope

In compliance with the One Health concept, it is important to look at the use of antibiotics as a whole, i.e. the use of both human and veterinary antibiotics together. According to the AWaRe classification of antibiotics for human use and the AMEG categorisation of antibiotics for use in animals, antibiotics are divided into different categories based on their resistance risk and medical importance. The same ingredient may be classified in the lowest risk category for humans and the risk for public health in veterinary medicine is estimated higher. The aim is comparing the selection of antimicrobial ingredients in treatment of humans by AWaRe classification and in treatment of companion animals by AMEG categorisation. This analysis includes data of human and veterinary antibiotics in pharmaceutical form as tablets.

Results and conclusions

In 2020–2024, 36 different antimicrobial ingredients were used in human treatment and 28 in companion animal treatment, 7 of them for both: Amoxicillin, Amoxicillin/clavulanic acid, Cefadroxil, Cefalexin, Clindamycin, Doxycycline, Metronidazole. Both financially and quantitatively, the most sold antibiotic for humans and companion animals was Amoxicillin/clavulanic acid, classified for humans by AWaRe classification as “Access” (green, first-line) and for animals by AMEG classification as “Caution” (yellow, limited use) category. Amoxicillin/clavulanic acid accounted for 23% of the total cost of human antibiotics and for 64% of the total cost of antibiotics used in treatment of companion animals. The overall sales of AWaRe category “Access” antibiotics accounted for 78% of total human antibiotics, and the sales of AMEG category “Caution” antibiotics accounted for 90% of all antibiotics used for companion animals, in Estonia.

AWaRe and AMEG classifications are intended as tools for optimizing and monitoring antibiotic use, also for monitoring the impact of stewardship policies implemented to manage antimicrobial resistance.

A23. In Search of Sensitive Indicators of River Environmental Health at the Catchment Scale

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Enterobacterale strains like *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter* spp. pose a serious epidemiological threat due to their potential as pathogenic organisms. Furthermore, bacteria belonging to the order Enterobacterales frequently exhibit resistance to carbapenem antibiotics and are capable of producing extended-spectrum β-lactamases (ESBLs). Consequently, they have been designated as critical priority pathogens by the World Health Organization (WHO, 2024).
Therefore, this study aimed to determine the impact of treated wastewater discharge on bacterial biodiversity within the order Enterobacterales and the presence of carbapenem resistance genes (CRGs) in river water samples at the catchment level.
River water was sampled at eight points from the upper Pilica River to its confluence with the Vistula (Poland). A total of 32 samples in four seasons were collected. Metagenomic DNA was extracted from the samples, and single-read Illumina sequencing was carried out. The number of CRGs in the samples was established through digital PCR. Physico-chemical parameters were studied in accordance with standard methods.
In the current study, the prevalence of Enterobacterales was lower than that of the other identified taxa. Enterobacterales accounted for about 1% of all amplicon sequence variants (ASVs), including Escherichia-Shigella (65.97% of Enterobacterales ASVs), *Yersinia* spp. (10.63%), *Klebsiella spp.* (9.69%), *Enterobacter spp.* (7.17%), *Serratia spp.* (4.21%) and others. All studied CRGs were present in the analysed samples. The most prevalent genes in river samples were bla_{VIM} and bla_{OXA}, while other studied genes (bla_{IMP}, bla_{NDM}, and bla_{KPC}) occurred at lower concentrations. The research confirmed a relationship between selected Enterobacterales ASVs and CRG concentrations in the analysed samples. Moreover, we observed a statistically significant relationship between the studied micropollutants and temperature, as well as BOD5, COD, total phosphorus and nitrogen.
Concentrations of all studied micropollutants, including CRGs, Enterobacterales ASVs, and physico-chemical parameters, were sensitive indicators of anthropogenic influence in river water.

A24. Investigating the Prevalence of Cefotaxime-Resistant *E. coli* and Antibiotic Resistance Genes in Drinking Water in Scotland

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The overuse of antimicrobials in humans and animals is driving the emergence of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs), which spread between the One Health compartments: humans, animals, and the environment (water, air, soil). The extent to which surface waters play a role in the dissemination of ARB/ARGs is poorly understood, but recreational use and drinking water may result in human exposure. This is the first large scale survey of the prevalence of cefotaxime-resistant *E. coli* (CTX-EC) and ARGs in drinking water sources and final water in Scotland. Drinking water sources with different catchments were selected to determine the effect of faecal pollution sources (human, livestock, agriculture, and wildlife) on CTX-EC and ARGs. Samples from 29 drinking water sources and three final waters (post-treatment) were analysed. Total *E. coli* and CTX-EC were enumerated and HTqPCR was used to quantify 56 ARGs.

CTX-EC were detected in seven drinking water sources (24%). In the majority (95%) of samples positive for CTX-EC, the proportion of CTX-EC to total *E. coli* was low (<2%). Logistic regression found significant associations between CTX-EC presence and two catchment variables: septic tanks and pastures. ARGs were present in 90% of drinking water sources and 44 ARGs from eight antibiotic classes were quantified; however, mean relative abundances of ARGs were low (3E-06 to 0.006 ARG copies per 16S rRNA gene). In final waters, all ARGs were below the limit of detection, and no *E. coli* or CTX-EC were recovered.

This study found that the overall AMR load in Scottish drinking water sources is low. CTX-EC concentrations were low, and their prevalence was around half of that found in coastal waters in Scotland. ARGs were widely detected in drinking water sources, but their relative abundances were low, and drinking water treatment processes effectively removed them.

A25. Extracellular and Intracellular Activity of Rifaximin α Against Bovine *Staphylococcus aureus*: Implications for Early Mastitis Therapy

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Subclinical mastitis caused by *Staphylococcus aureus* remains a major challenge due to persistence, recurrence and antimicrobial use. Bacterial adaptation to mammary gland conditions and intracellular survival contribute to therapeutic failure. Rifaximin α (RIFα), a semisynthetic rifamycin widely used for intramammary therapy, exhibits strong antibacterial activity; however, an integrated pharmacodynamic evaluation considering both extracellular and intracellular compartments is limited.

In vitro pharmacodynamic studies were performed to characterize the extracellular and intracellular activity of RIFα against bovine *S. aureus*. Extracellular activity was assessed using minimum inhibitory concentration determination, post-antibiotic effect, and time–kill curves under mammary-simulated conditions, including different pH values (7.4, 6.5 and 5.0) and media supplemented with bovine serum or milk. Antibacterial activity was quantified using the antibacterial effect index (E). Intracellular activity was evaluated in polymorphonuclear cells (PMNs) isolated from bovine blood. PMNs were infected with *S. aureus* ATCC 29213, extracellular bacteria were eliminated, and infected cells were exposed to RIFα at 3×MIC and 10×MIC. Intracellular viable bacteria were quantified by colony-forming unit enumeration following cell lysis.

RIFα demonstrated potent extracellular activity, achieving stable bactericidal effects ($E \leq -3$) at 4–8×MIC across pH conditions and in the presence of milk or serum, confirming high antibacterial efficacy under mammary-relevant conditions. In contrast, intracellular exposure resulted in only partial reductions in intracellular *S. aureus*, with decreases of approximately 1.5–1.7 $\log_{10}$ CFU after 8 h, without reaching bactericidal thresholds.

From a PK/PD standpoint, the contrast between potent extracellular bactericidal activity and limited intracellular efficacy underscores the importance of early exposure-driven antimicrobial action. These findings emphasize the value of dynamic pharmacodynamic approaches that integrate both extracellular and intracellular compartments to better inform rational dosing strategies against persistent *Staphylococcus aureus* infections.

A26. A One Health Assessment of Antimicrobial Resistance Genes in Escherichia coli from Laguna de Bay, Its Tributary Rivers, and Surrounding Backyard Farms in the Philippines

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Laguna de Bay, the largest lake in the Philippines, is fed by 21 tributary river systems and serves as a multi-use water resource for economic and recreational purposes. Backyard farms are also prevalent in the communities around the lake. Unregulated use of antibiotics in these farms and local communities contributes to the emergence of antimicrobial-resistant (AMR) bacteria, posing a significant threat to public health and the environment. While there are existing studies on AMR in the Philippines, research on the prevalence of bacteria harboring antimicrobial-resistance genes (ARGs) in environmental waters and animals remains limited. Therefore, this study aimed to determine the antibiotic resistance profiles of Escherichia coli isolated from selected stations of Laguna Lake, 11 surrounding tributary rivers, and backyard farms near the lake. Environmental water and fecal samples were collected from these sites, and a total of 5,051 E. coli were isolated and genotypically screened for the presence of ARGs: β-lactamase (blaCTX-M, blaSHV, blaTEM, and blaOXA-1), carbapenemase (blaNDM), sulfonamide (sul1 and sul2), and tetracycline (tetA). Among these, tetA was the most frequently detected gene in both environmental water (31%) and animal hosts (41%), followed by blaTEM (27%; 32%), sul2 (16%; 19%), sul1 (9%; 5%), blaSHV (4%; 2%), and blaCTX-M (2%; 2%). blaOXA-1 and blaNDM had lower detection frequencies (>1%) in environmental water and were absent in any animal hosts. Among isolates harboring at least two ARGs, the blaTEM/tetA (n=405) combination was the most frequently detected in water samples. In animals, tetA/blaTEM gene combination was most frequent in poultry and swine, while tetA-blaTEM-sul2 combination dominated in ducks. Overall, the results confirm the presence of ARGs in E. coli isolates from Laguna de Bay, its tributaries, and surrounding farms, which can be used as a reference to monitor environmental contamination and mitigate the spread of antimicrobial resistance.

A27. Isolation and Characterization of Peptide molecules from *Levilactobacillus brevis* and their application as Antimicrobials

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The burden of increased antimicrobial resistance in microorganisms has complicated disease management and healthcare systems. Mechanisms like enzymatic modification of antibiotics, biofilm formation and genetic mutations enable pathogens to evade antibiotic treatments. In this view, alternatives for antimicrobial agents from natural sources are a subject of concern and should be explored immediately. Bacteriocins are antimicrobial peptides with targeted activity against specific bacteria. They are effective at low concentrations (Soltani *et al.,* 2022) and demonstrate both narrow- and broad-spectrum activity. Bacteriocins selectively target and inhibit pathogenic bacteria, minimizing the development of resistance and preserving beneficial microbiota through quorum sensing. Their molecular weight ranges from <5 to 80 kDa with a smaller number of amino acids according to RiPP nomenclature of bacteriocin classification. Our study has emphasized isolation of novel peptides from the species of *Levilactobacillus brevis* through microbiological techniques and their purification using Prep-HPLC and characterization by biomolecular techniques—High-Performance Thin-Layer Chromatography (HPTLC), Proton-Nuclear Magnetic Resonance (H-NMR) and MALDI-TOF. The antimicrobial activity of purified bacteriocin was determined using an agar-well assay against pathogenic organisms—*Streptococcus mutans*, *Escherichia coli* and *Candida auris.* The isolated peptide is characterized as a Class II bacteriocin with amino acid no. 8 (partial N-terminal sequence) and size 0.86 kDa (in accordance with RiPP nomenclature of bacteriocin classification). Peptides displayed potential antimicrobial effects with zones of inhibition against *Escherichia coli* at 0.2 cm, *Streptococcus mutans* at 0.3 cm and *Candida albicans* at 0.12 cm; p value = 0.00004 ($p \leq 0.05$). This is statistically significant. There is a current need to explore other such peptides and further apply them in pharmacological, food and biomedical sectors to strengthen their effectiveness against antimicrobial resistance. The current research data adds to the need to search for such alternate molecules.

A28. Polymeric Nanoparticle-Mediated Enhancement of Cefuroxime Axetil: Potent Antibiofilm Activity Against Biofilm-Forming Pathogens

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Biofilm-driven infections represent a critical barrier to effective antimicrobial therapy, contributing significantly to the global burden of antibiotic resistance. Overcoming the limited biofilm penetration, rapid drug clearance, and suboptimal local antibiotic concentrations associated with conventional formulations remains a key therapeutic challenge. In this context, cefuroxime axetil-loaded polymeric nanoparticles based on Eudragit RS100 and RL100 were designed to enhance antibacterial and antibiofilm activity. Polymeric nanoparticle systems offer several advantages, including improved drug stability, enhanced penetration into the biofilm matrix, controlled and sustained drug release, and increased local drug concentration at the site of infection. Antibacterial activity was evaluated against clinically relevant biofilm-forming pathogens (Staphylococcus aureus ATCC 29213 and Escherichia coli ATCC 35218), revealing minimum inhibitory concentration (MIC) values ranging from 500 to 15.62 μg/mL. Importantly, the nanoparticulate system demonstrated pronounced antibiofilm efficacy across all tested concentrations. At 500 μg/mL, biofilm inhibition exceeded ≥94±1.48%, indicating near-complete suppression of biofilm formation. Remarkably, substantial inhibition ≥75±1.00% was retained even at the lowest concentration tested (15.62 μg/mL), underscoring the robustness of the formulation. The enhanced antibiofilm activity is likely driven by improved antibiotic diffusion into the biofilm matrix combined with sustained drug release characteristics of the Eudragit polymers, enabling prolonged antimicrobial exposure. Collectively, these findings position Eudragit-based cefuroxime axetil nanoparticles as a robust and promising platform to combat biofilm-associated infections and antimicrobial resistance. This approach highlights the potential of nanoparticle-mediated antibiotic delivery systems for next-generation therapeutic strategies targeting persistent infections.

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A29. Oneperu: Peruvian Bioinformatics Platform To Contribute To The Fight Against Antimicrobial Resistance

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ONEPERÚ is a bioinformatics platform developed for the genomic surveillance of antimicrobial-resistant (AMR) bacteria, focused on *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. It integrates phenotypic, genomic, epidemiological, and clinical data obtained from human, environmental, and companion animal samples, enabling the analysis of resistance patterns and supporting public health decision-making under the UNASALUD (One Health) approach.
During the first year (June 2024 – June 2025), the platform was fully developed, including its architecture, design, maps, tables, and detailed sample module. Likewise, technical and functional requirements were collected, the platform capacity was expanded to include four microorganisms, and data upload and verification procedures were implemented and validated.
In the second year (July 2025 – present), 115 complete genomes were downloaded in FASTA format from the NCBI/GenBank repository, corresponding to *E. coli* (30), *K. pneumoniae* (30), *P. aeruginosa* (30), and *A. baumannii* (25). A script was developed to automate genome download and genomic analysis, recording variables such as source, accession number, and genome characteristics to determine the resistome, efflux pump genes, virulome, serotypes, phylogroups, sequence types, plasmids, fimbriae, and k-locus.
In parallel, 83 samples were sequenced using MinION technology (Oxford Nanopore), obtained from 75 clinical MDR isolates, 4 companion animal isolates and 4 environmental isolates. From FASTQ files, high-quality complete genomes were assembled, enabling in-depth characterization of the resistome and virulome, as well as the identification of high-risk clones such as ST-486 of *P. aeruginosa* and ST11–KL111 of *K. pneumoniae*, reported for the first time in the country.
Finally, future work will continue the genomic processing of 62 new environmental and companion animal isolates using MinION technology, using previously developed scripts for their analysis.

A30. Integrating Competitive Bioassays and Mechanistic Models to Assess Environmental AMR Selection

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Antimicrobial resistance (AMR) is increasingly recognised as a significant health challenge, driven not only by the clinical use of antibiotics but also by the dissemination of antibiotic residues and resistance genes (ARGs) through wastewater systems and natural ecosystems. Spatially explicit exposure models, such as ePiE, quantify antibiotic concentrations in surface waters; however, current environmental AMR risk assessment methods rely on simplified assumptions about microbial community responses. Existing approaches, such as the 8-day SELECT bioassay, typically infer selection from growth inhibition thresholds without explicitly capturing differential growth dynamics between susceptible and resistant bacteria, offering limited mechanistic insight into how AMR selection emerges under realistic environmental exposure scenarios.

In this study, we aim to bridge this gap by integrating experimental and modelling approaches. First, we will adapt an *in vitro* growth inhibition bioassay into a co-culture competition assay that simultaneously exposes fluorescently labelled wild-type and ciprofloxacin-resistant *Escherichia coli* strains to antibiotics. By quantifying shifts in relative abundance, this assay will provide a direct and time-efficient measure of AMR selection pressure. Second, we will employ data from both current and adapted assays to develop mechanistic *in silico* models describing antibiotic-dependent growth inhibition for susceptible and resistant strains. These models will generate validated rules for bacterial growth and competition, supporting the development of agent-based simulations of environmental microbial communities.

Together, this work will establish an experimentally grounded and mechanistically informed framework for evaluating AMR selection in environmental contexts, enabling more robust prospective risk assessments for antibiotics and supporting stronger environmental protection strategies.

A31. Evaluating the Influence of Interactions Among Selected *Enterobacteriaceae* on Antibiotic Resistance Profiles

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Interspecies relationships within microbial consortia are often associated with mixed microbial infections and are known to impact bacterial resistance to antibiotics. This research examined the interactions among *Escherichia*, *Proteus*, and *Klebsiella* species on antibiotic resistance profiles. Single and mixed cultures of the bacterial isolates were prepared through combinations of two, three and four species. Antibiotic susceptibility testing for mono-cultures was performed by lawn inoculation of Mueller–Hinton agar plates using standardized bacterial suspensions. Gram-negative antibiotic discs were placed on the inoculated plates, which were then incubated at 37 °C for 24 h for mono-culture and mixed-culture plates. Following incubation, zones of inhibition were measured, and compared to evaluate differences in antibiotic susceptibility between single and mixed cultures. *E. coli* (PX588540) and *Kl. pneumoniae* (PX588542) exhibited intermediate susceptibility to amoxicillin clavulanate, whereas *E. coli* (PX588541) and *P. mirabilis* (PX588543) were resistant. *E. coli* (PX588541) and P. mirabilis (PX588543) conferred resistance to other members of the mixed culture. In mono-culture assays, both *E. coli* (PX588540) and *E. coli* (PX588541) were resistant to cefotaxime; however, an intermediate response was recorded during co-culture. Resistance to gentamicin in mono-culture was observed only in *P. mirabilis* (PX588543). *Kl. pneumoniae* (PX588542) was inhibited by nalidixic acid in mono-culture; it demonstrated tolerance to the antibiotic across all co-culture combinations. Additionally, *E. coli* (PX588541) displayed intermediate susceptibility to nitrofurantoin but became susceptible when co-cultured with *E. coli* (PX588540). These findings demonstrate that bacterial isolates exhibit different susceptibility and resistance profiles in co-culture compared with pure cultures.

A32. Progress on CPD 14, A Potential Bam Complex Inhibitor

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The β-barrel assembly machinery (Bam complex) is essential for folding and inserting outer-membrane proteins (OMPs) in Gram-negative bacteria (GNB) and represents a structurally conserved antibacterial target. In a previous screen for OM disruption, CPD14 exhibited phenotypes consistent with Bam perturbation, including reduced porin abundance, hypersensitivity of envelope biogenesis mutants, robust activation of envelope-stress reporters typical of impaired OMP assembly, and inhibition in a Bam functionality assay. But, limited availability of CPD14 restricted definitive target validation.

CPD14 was synthesized in collaboration with consortium partner to enable validation. CPD14-resistant *E. coli* isolates were generated by stepwise selection at increasing compound concentrations, and whole-genome sequencing of resistant mutants identified a single nucleotide mutation. Genomic analysis identified a single-nucleotide substitution in *basS*, encoding the histidine kinase of the BasSR two-component system, in CPD14-resistant isolates. BasS modulates lipid A remodelling and outer-membrane charge; disruption of BasSR signalling can alter OM integrity and envelope stress responses. To be noted, is that resistance appeared to arise via a specific, low-frequency route: while initial CPD14 resistance mapped to *basS*, subsequent selections did not reproduce the mutation, suggesting limited accessible resistance pathways and possible alternative or compensatory mechanisms.

A panel of 30 CPD14 variants were synthesized and evaluated for antibacterial activity against *E. coli, B. subtilis, and S. aureus* to determine specificity to GNBs. Phenotypic assays included an envelope-stress (Rcs) response readout, minimum inhibitory concentration (MIC) determinations, and haemolysis assays to assess selectivity and toxicity. Among the variants, a few displayed significantly enhanced antibacterial activity relative to CPD14, providing productive starting points for SAR optimization. Ongoing work focuses on identifying variants with preserved GNB specificity, as these are more likely to engage Bam, the primary target in this context.

These findings establish a starting framework for CPD14's mechanism of action, guiding the refinement of Bam-targeting antibacterial compounds.

A33. Drug Resistance of *M. tuberculosis* to Pyrazinamide Among Drug-Resistant Clinical Isolates, Including MDR/XDR Strains in Kazakhstan

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Kazakhstan is among the 30 countries worldwide with the highest rates of MDR-TB. Pyrazinamide is an anti-TB drug that plays an important role in shortening the duration of therapy because it targets persistent bacilli and enhances the effectiveness of combination regimens.
The aim of the study was to determine the frequency of pyrazinamide resistance among drug-resistant (DR) *M. tuberculosis* isolates from different regions of Kazakhstan and to identify genetic families.
271 drug-resistant clinical isolates of *M. tuberculosis* were collected, of which 143 (52.8%) were multidrug-resistant, 79 (29.2%) pre-XDR, 21 (7.7%) monoresistant, 19 (7.0%) polyresistant, and 9 (3.3%) XDR. All clinical isolates were tested for drug susceptibility to first/second-line anti-TB drugs and pyrazinamide (PZA) using the BACTEC MGIT-960. All clinical isolates of *M. tuberculosis* were spoligotyped on the Zeesan automated analysis system, SLAN-96 (Zeesan Biotech, China), using the MeltPro® Mycobacterium Tuberculosis McSpoligotyping kit.
Of the 271 drug-resistant clinical isolates of *M. tuberculosis*, 158 (58.3%) were phenotypically resistant to pyrazinamide, whereas 113 (41.7%) were susceptible. Spoligotyping results showed a predominance of the Beijing genotype among both pyrazinamide-resistant (n = 135, 85.4%) and pyrazinamide-susceptible (n = 90, 79.6%) clinical isolates of *M. tuberculosis*.
Analysis of the distribution among drug-resistant isolates showed that among 143 (100%) MDR isolates, 73 (51.0%) were identified as resistant to pyrazinamide, among 79 (100%) pre-XDR isolates, 63 (69.7%) were resistant to pyrazinamide, and among 9 XDRs, 7 were resistant to pyrazinamide. Among 19 polyresistant isolates, 9 (47.4%) were resistant to pyrazinamide. Among all drug-resistant clinical isolates, 225 (83%) were the Beijing genotypes, and 46 (17.0%) were non-Beijing genotypes represented by LAM, T, URAL, H, S, MANU-2, and Unknown.
Among multidrug-resistant and polyresistant strains, nearly half are susceptible to pyrazinamide (49.0% and 52.6%, respectively), which allows PZA to be included in the treatment regimen. PZA kills persistent, slow-growing bacilli that survive standard therapy, preventing relapse, which makes PZA especially effective. Genotyping results showed that Beijing genetic lines continue to dominate and play a key role in the spread of drug-resistant tuberculosis, including drug resistance to pyrazinamide in Kazakhstan.

A34. One Health Surveillance of Environmental *Escherichia coli* and Antimicrobial Resistance in Broiler Production Systems in Morocco

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Background: Broiler production environments can act as reservoirs for Escherichia coli and antimicrobial resistance (AMR), facilitating dissemination through manure, soil, and water interfaces. However, matrix-resolved environmental data remain limited in North Africa.

Objectives: To estimate the prevalence of E. coli across key broiler-farm environmental matrices and to characterize phenotypic AMR, including ESBL production and multidrug resistance (MDR), among recovered isolates.

Methods: A cross-sectional survey was conducted in 13 broiler farms in the Rabat–Salé–Zemmour–Zaer area (Morocco) from 24 November 2023 to 5 May 2025. A total of 506 samples were collected from poultry manure, drinking troughs, soil (on-farm), soil at 200 m and 500 m from farms, and well water. Solid samples (25 g) were pre-enriched in buffered peptone water (25 g + 225 mL) and plated on EMB agar; well water was analyzed by membrane filtration (100 mL; 0.45 µm). Identification was confirmed using API 20E. Antimicrobial susceptibility testing was performed by Kirby–Bauer disc diffusion against 20 antibiotics (CLSI 2020). ESBL phenotype was assessed by double-disc synergy/combination testing and confirmed on Brilliance™ ESBL agar. Prevalence and susceptibility proportions were reported with 95% exact binomial confidence intervals (R). MDR was defined as resistance to ≥1 agent in ≥3 antimicrobial classes.

Results: Overall, *E. coli* was detected in 190/506 samples (37.5%, 95% CI: 33.3–41.9). Prevalence was highest in poultry manure (48.1%) and soils (38.0–44.3%), and lowest in well water (23.2%). Among 190 isolates, resistance was highest to ampicillin (84.7%), tetracycline (61.6%), amoxicillin (56.8%), and chloramphenicol (55.8%), with notable resistance to nalidixic acid (44.7%), trimethoprim–sulfamethoxazole (36.3%), and ciprofloxacin (32.6%). Resistance to carbapenems was rare (0–0.5%), and susceptibility to cefepime and piperacillin–tazobactam was near-complete. ESBL phenotype was detected in 35/190 isolates (18.4%), and MDR was observed in 110/190 isolates (57.9%).

Conclusion: *E. coli* and clinically relevant AMR phenotypes (ESBL and MDR) were widely distributed across broiler-farm environmental matrices, particularly manure and soils, supporting the need for strengthened antimicrobial stewardship and targeted environmental risk management within a One Health framework.

A35. Gender and Equity as Social Determinants of Antimicrobial Resistance (AMR) in Malaysian Shrimp Aquaculture: A One Health Study

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Shrimp aquaculture is one of Malaysia's most rapidly expanding food production sectors, contributing significantly to national food security and export revenue. Despite its economic importance, the industry faces persistent challenges related to disease outbreaks, environmental degradation, and inappropriate antimicrobial use (AMU), which contributes to the global threat of antimicrobial resistance (AMR). Existing interventions have largely emphasized technical and regulatory solutions, with limited attention to the social dynamics shaping farm-level antimicrobial practices. This study addresses this gap by examining how gender and equity-related factors influence AMU and biosecurity practices in Malaysian shrimp aquaculture. Using a qualitative research design and data triangulation, the study draws on semi-structured interviews and focus group discussions conducted across 18 shrimp farms of varying scales (small, medium, and large) in six regions of Malaysia, including East Malaysia (Sabah and Sarawak). Participants included 18 farm owners and managers and 36 farm workers, selected to reflect diverse sociocultural, geographic, and organizational contexts. Analysis focused on the intersection of gender, socioeconomic status, education, nationality, seniority, and cultural norms in shaping AMR-related practices. The findings reveal that farm tasks and decision-making processes are strongly gendered and hierarchical. Men typically dominate decisions regarding antimicrobial application, while women and interns are often relegated to administrative or supportive roles with limited authority. Migrant workers, who comprise a substantial portion of the workforce, face restricted access to training, information, and decision-making power related to AMU and biosecurity. These social inequities significantly influence antimicrobial practices, exposure risks, and the distribution of benefits and responsibilities. The study demonstrates that integrating gender and equity perspectives is essential for developing inclusive, context-sensitive, and sustainable AMR mitigation strategies aligned with Malaysia's national AMR framework.

A36. Isolation of Multi-Resistant Bacteria to Antibiotics of the ESKAPE Group from Hospital Wastewater and the WWTP of Panama

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Antimicrobial resistance (AMR) poses a global challenge, particularly with regard to the ESKAPE pathogens: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp., which are associated with severe and often fatal infections. This study aimed to assess the occurrence of ESKAPE pathogens and Serratia marcescens in hospital wastewater effluents and influent samples from the wastewater treatment plant (WWTP) in Panama City. The methodology involved enriching cell cultures, utilizing selective media for growth, and conducting biochemical assessments using the Vitek® 2 automated system for detection and susceptibility profiling. Molecular confirmation of resistance genes was achieved through end-point PCR analysis. A total of 75 strains each of A. baumannii and P. aeruginosa, 50 strains of S. aureus, 45 strains of Enterococcus sp., 10 strains of S. marcescens, and 75 strains of Klebsiella spp. were identified. In terms of genotypic profiles, it was found that 3% of the P. aeruginosa strains possessed the blaVIM gene, while a significant majority, 97%, carried blaOXA-48. Among Klebsiella spp., 13% contained blaKPC, 3% had blaNDM, 13% showed presence of blaCTX-M, 6% had blaVIM, and 8% were positive for the pilV gene. Additionally, the mecA gene was identified in 20% of S. aureus strains classified as MRSA. These findings underscore the significance of wastewater as a crucial reservoir for resistance genes and highlight a major public health concern in Panama.

A37. *Pseudomonas Aeruginosa* Extracellular Vesicles as Carriers of Antimicrobial Resistance and Virulence Factors

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Bacterial infections remain a major global health challenge, aggravated by the increasing prevalence of multidrug-resistant (MDR) pathogens. The rapid dissemination of carbapenem resistance mediated by metallo-beta-lactamases, such as VIM, threatens the effective treatment of *Pseudomonas aeruginosa* infections. Growing evidence indicates that bacterial extracellular vesicles (EVs) contribute to the horizontal transfer of antimicrobial resistance and virulence determinants, although this mechanism remains poorly characterised. This study aimed to analyse and characterise EVs produced by high-risk clones as carriers of resistance and virulence factors.
Two clinical MDR *P. aeruginosa* strains belonging to high-risk clone ST175 (Ps56 and Ps713) and their respective mutant Δbla_{VIM} were included. EVs were recovered from culture supernatants by centrifugation and purified by filtration and using the ExoBacteria™ OMV Isolation Kit. Vesicle size and concentration were assessed by Nanoparticle Tracking Analysis and Transmission Electron Microscopy (TEM), protein content was quantified by Bradford assay, and proteomic profiling was performed by data-independent acquisition mass spectrometry. EV-associated DNA (treated with/without DNase) and bacterial genomes were analysed by PCR and whole-genome sequencing (Illumina HiSeq2500 and Nanopore PromethION). Carbapenemase activity was evaluated using NG-Test Carba5.

EVs displayed diameters of 125-145nm and concentrations of 1.52×10^{8}-2.66×10^{8} particles/mL, with protein levels of 1436-1592µg/mL. TEM showed vesicles with a single membrane (outer membrane vesicles) and vesicles with a double membrane (outer and inner membrane vesicles). The bla_{VIM-2} gene was detected in Ps713 EVs within an In56 integron on a plasmid, while bla_{VIM-20} was chromosomally located in Ps56. Both EVs exhibited carbapenemase activity. Proteomic analysis showed enrichment of functional categories related to translation, ribosomal structure, nucleotide and coenzyme transport and metabolism. Multiple virulence factors were detected by proteomic analysis, highlighting toxins such as ExoA and quorum-sensing factors such as Pqs. These findings support a critical role for EVs in *P. aeruginosa* pathogenicity and dissemination of antibiotic resistance.

A38. Antimicrobial Resistance, Virulence and Genetic Diversity of *Escherichia Coli* From a Hospital Food Service

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Background: *Escherichia coli* is an indicator of food contamination and an important public health pathogen with high ability to acquire virulence and antimicrobial resistance. The presence of potentially pathogenic strains in the food chain poses a high risk to consumers, particularly in highly vulnerable environments such as hospitals. This study aims to investigate the antimicrobial resistance profiles, molecular typing, virulence and integrons of fifteen *E. coli* isolates recovered from a hospital kitchen.

Material and methods: Fifteen *E. coli* strains were isolated from different raw foods (n=10) and processing areas (n=5) in a hospital kitchen. Molecular typing was assessed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Antimicrobial susceptibility and phenotypes of ESBL, AmpC, and carbapenemase were tested by disc diffusion methods. The molecular characterisation (serotype, phylogeny, virulence factors and presence of integrons) was performed by PCR.

Results: Eleven PFGE patterns and ten sequence types (STs) were identified among the fifteen isolates, which included high-risk clones belonging to ST10 Cplx (ST10, n=1) and ST155 Cplx (ST58, n=1). No O25a, O25b or O157 serotypes were detected. Phylogroups were distributed as A (n=4), B1 (8), B2 (2), and E (1). Antimicrobial resistance analysis determined that 47% were multidrug resistant strains. Four strains were ESBL producers associated with the bla_{SHV-12} gene, four strains showed an AmpC phenotype, and no carbapenem-resistant *E. coli* was observed. Class 1 integrons were detected in 20% of isolates, carrying *dfrA1–aadA1* or *dfrA12–gcuF–aadA2* arrangements. Concerning virulence-associated factors, all isolates were positive for the *fimA* gene, and 53% of the isolates carried the *aer* gene.

Conclusions: The high genetic diversity, the presence of antimicrobial resistance and virulence determinants among *E. coli* isolates from a hospital food service environment underscore a potential threat to food safety and public health.

A39. China's Response to Antimicrobial Resistance Governance Practices, 2017-2024: A Systematic Gap Analysis Based on Artificial Intelligence

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Background
Antimicrobial resistance (AMR) is a major global public health threat. Yet, existing studies lack a systematic review of cross-sectoral AMR governance. This study aims to comprehensively assess China's sectoral responses to AMR from 2017-2024, analyze sectoral and regional governance differences, and quantify correlations between response levels and AMR indicators.

Methods
We used AI (e.g., the collaborative Scrapy web crawling technique) to collect text data on how Chinese government departments at all levels have responded to AMR from 2017 to 2024, including policy papers, news, official WeChat posts, and other public data. A Large Language Model standardized the data for sectoral and regional comparison. Finally, we used a distribution-lag model to evaluate the correlations between provincial response levels and AMR.

Results
A total of 11,291 datapoints mapped China's sectoral AMR governance, with human (54.5%), animal (39.5%), environmental (4.2%), and "One Health" (1.6%) sectors contributing. From 2017 to 2024, the number of relevant texts included increased steadily, peaking in 2020 before declining. The main content types of the included texts were administrative management and policy guidance, at 42.6% and 18.7%, respectively, while basic support (8.7%), industrial innovation (7.3%), academic exchange (5.6%), and international cooperation (0.5%) accounted for relatively low proportions. AMR governance response varied by province, higher response scores correlated with lower prevalence.

Conclusions
Environment sector has begun designing policies, yet monitoring remains challenging. Improving One Health awareness among the public and regional leaders is key to action, with cross-sectoral education promising, especially in rural areas. We call for regional pilots for sharing human-animal surveillance data to forecast AMR better.

Limitations
First, restricted internal access to some data prevented full collection, which to some extent reflects the transparency of governance. Second, despite pre-defined categorization and thorough reviewer communication with third-party adjudication, textual data complexity may still cause minor classification discrepancies among different researchers.

Poster Session B

B01. Phytocompounds Present in Essential Oils Used as Alternative Antimicrobial Agents Against *Escherichia Coli* O157:H7

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Escherichia coli O157:H7 is an enterohemorrhagic pathogen responsible for severe gastrointestinal illness, including hemorrhagic colitis and hemolytic uremic syndrome (HUS), with disease severity primarily driven by Shiga toxin production and other virulence determinants. Conventional antibiotic therapy is often contraindicated due to toxin induction, intensifying the interest in traditional medicinal plants and their bioactive constituents as alternative therapeutic options.

In this study, we investigated the antibacterial activity of three essential oil-derived phytocompounds widely used in ethnomedicine: geraniol from *Cymbopogon martini*, cinnamaldehyde from *Cinnamomum verum* bark oil, and citral from *Cymbopogon flexuosus*. These compounds are traditionally valued for their antimicrobial and gastrointestinal protective properties. Antibacterial efficacy against *E. coli* O157:H7 was evaluated using growth inhibition assays and time-kill analyses.
All three phytocompounds demonstrated concentration-dependent inhibition of bacterial growth. Time-kill assays revealed significant reductions in viable bacterial counts at sub-minimal inhibitory concentrations, indicating predominantly bacteriostatic activity. Ongoing real-time PCR studies aim to assess the influence of these compounds on the expression of key virulence-associated genes.
The present findings support the ethnopharmacological relevance of *Cymbopogon* spp. and *C. verum*-derived phytochemicals and highlight their potential as natural antibacterial agents against enterohemorrhagic *E. coli*. These results provide a scientific basis for further exploration of traditionally used essential oil components in the development of alternative strategies for managing enteric infections.

B02. A Stepwise Diagnostic Stewardship Intervention for Ordering Urine Cultures in Saudi Arabia: A Prospective Pre- and Post-Implementation Study

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Introduction: Inappropriate urine culture testing can lead to unnecessary antibiotics use and antimicrobial resistance. We evaluated the implementation of a stepwise stewardship intervention, with a focus on the appropriateness of urine culture orders and antibiotic use.Methods: We conducted a quasi-experimental study from August 2021 to Jun 2023 at a teaching hospital in Riyadh, Saudi Arabia. We included adult patients with urine culture orders. A stepwise antimicrobial stewardship intervention was implemented in two phases. In the first phase, a clinical decision support (CDS) tool was introduced in February 2022. In the second phase, a reflux urine culture was implemented in January 2023. Our outcomes were the percentage of inappropriate urine cultures ordered and antibiotic use. Outcomes were assessed at baseline, after the CDS intervention, and after the reflux urine culture intervention. We used multivariable logistic regression to estimate adjusted odds ratio, controlling for potential confounding variables. Results: During the pre-intervention period, the percentage of inappropriate urine culture order was 41.6%. Following the implementation of the first intervention—CDS—the percentage decreased to 36.4%. Subsequently, with the addition of reflux urine culture in the second phase of the stepwise intervention, the percentage further decreased to 28.6% (p value=<0.001). The stepwise intervention was associated with 15 lower odds of inappropriate urine culture ordering (adjOR 0.85; 95%CI 0. 0.69 – 0.90; p = 0.027). A significant reduction in Unnecessary antibiotics was observed across the three phases: 72.9% pre-intervention, 85.7% post-CDS, and 25% post-CDS and reflux urine culture (p <0.0001). The stepwise intervention was associated with 75% reduction in unnecessary antibiotic use (aOR 0.25; 95% CI 0.17 – 0.37, p <0.001)

Conclusions: This stepwise intervention suggest that each intervention contributed incrementally to decrease inappropriate urine culture and unnecessary antibiotic use.

B03. In Vitro Activity of Eravacycline Against Cefiderocolresistant ESBL/NDM-producing Enterobacterales: A Preliminary Series

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Introduction
Infections caused by carbapenemase-producing Enterobacterales, represent a major therapeutic challenge. Among them, NDM-type metallo-β-lactamases limit treatment options. Cefiderocol has emerged as one of the main therapeutic alternatives.
Eravacycline, a novel fluorocycline approved for complicated intra-abdominal infections, has demonstrated potent in vitro activity against carbapenemase-producing Enterobacterales, including KPC, NDM, and OXA-48 producing isolates. However, its clinical use should be interpreted in light of infection source, drug exposure, absence of a urinary indication, and the limited availability of outcome data in infections caused by cefiderocol-resistant NDM-producing organisms.
Objectives
To evaluate the in vitro activity of eravacycline against cefiderocol-resistant ESBL/NDM-producing Enterobacterales and to describe tetracycline-associated resistance determinants detected by whole-genome sequencing.
Materials and methods
The MIC of eravacycline was determined using gradient diffusion (Etest, bioMérieux, France), and cefiderocol MIC was determined using a commercial broth microdilution system (UMIC, Bruker, USA), in nine ESBL- and NDM-producing Enterobacterales isolates (six *Escherichia coli* and three *Klebsiella pneumoniae*). Four isolates were recovered from urine, four from rectal surveillance swabs, and one from prosthetic tissue.Isolates were sequenced by Oxford Nanopore Technologies.
Results
All nine ESBL/NDM-producing Enterobacterales isolates showed low eravacycline MICs according to the interpretive criteria applied in this study, while all were classified as cefiderocol-resistant. Genomic analysis identified multiple tetracycline-associated resistance determinants, including efflux systems or related components (acrAB-tolC, oqxAB, KpnEF, tet(A), tet(B)), transporters (emrKY), and regulatory genes (marA, evgAS). Despite the presence of these determinants, no phenotypic reduction in eravacycline activity was observed in this isolate set.
Conclusions
Eravacycline showed high in vitro activity against ESBL/NDM -producing Enterobacterales with cefiderocol resistance. The presence of tetracycline resistance genes was not associated with reduced susceptibility to eravacyline, suggesting its ability to overcome classical resistance mechanisms. These findings are clinically relevant as a hypothesis-generating observation in difficult-to-treat NDM-producing Enterobacterales.

B04. Genomic Co-Occurrence of blaNDM-5 and Yersiniabactin-Associated Loci in Cefiderocol-Resistant Enterobacterales

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Introduction
Resistance to cefiderocol (CFDC) among Gram-negative bacteria is multifactorial and may involve beta-lactamase activity, metallo-beta-lactamase production, alterations in siderophore uptake pathways, changes in permeability, efflux, and/or target-related mechanisms.
CFDC is a siderophore-conjugated cephalosporin with a catechol residue that allows iron binding and facilitates entry through bacterial iron acquisition systems. Therefore, the expression and functionality of iron uptake receptors may influence CFDC activity.
Several siderophore systems, including enterobactin, aerobactin, yersiniabactin and salmochelin, may be relevant under iron-limited conditions, although their contribution to CFDC susceptibility depends on bacterial species, receptor integrity, gene expression and co-existing resistance mechanisms.

Objectives
To describe whole-genome sequencing findings in CFDC-resistant NDM-producing Enterobacterales, focusing on the co-occurrence of blaNDM-5 and genes involved in siderophore/iron acquisition pathways, and to discuss their potential contribution to the observed CFDC-resistant phenotype.

Materials and methods
Five CFDC-resistant NDM-producing Enterobacterales isolates (CFDC MIC range: 4-16 mg/L) were analyzed by whole-genome sequencing. The isolates carried blaNDM-5, and genes associated with the yersiniabactin system, including irp1 and fyuA, were identified in all cases.
In addition, other siderophore-associated genes were detected in some isolates.

Results
All five CFDC-resistant NDM-producing isolates carried blaNDM-5 and the yersiniabactin-associated genes irp1 and fyuA. Additional siderophore-related genes were variably present across isolates, suggesting heterogeneity in iron acquisition repertoires.
The consistent co-occurrence of blaNDM-5 with yersiniabactin-associated loci is biologically plausible in the context of reduced CFDC activity.

Conclusions
These preliminary WGS data show a consistent co-occurrence of blaNDM-5 and yersiniabactin-associated genes among CFDC-resistant Enterobacterales isolates. The findings support the hypothesis that siderophore/iron uptake pathways may contribute to CFDC resistance in NDM-5 producers, but functional studies and comparative genomic analyses are required to establish causality.

B05. Hybrid Biomaterial Based on PCL, Mn-HA and Inulin-g-bPEI-PLA: Evaluation of its Antimicrobial Potential

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Bone regeneration is frequently compromised by post-surgical infections and the growing prevalence of antimicrobial resistance, underscoring the urgent need for biomaterials with intrinsic, non-leaching antibacterial properties that can mitigate the emergence of resistant strains (Sadowska et al., 2021). In this study, a multifunctional hybrid biomaterial composed of polycaprolactone (PCL), manganese-doped hydroxyapatite (Mn-HA), and the amphiphilic copolymer inulin-g-branched polyethyleneimine-g-poly(D,L-lactide) (INU-bPEI-PLA) (Mazzacano et al., 2025) was developed and evaluated for its antimicrobial potential. PCL was selected as the structural matrix owing to its established biocompatibility, bioresorbability, mechanical stability, favorable processing characteristics, and controlled degradability (Tommasino et al., 2025). Mn-HA was incorporated as a bioactive inorganic phase capable of enhancing osteogenic responses (Bauer et al., 2024), while released Mn^{2+} ions have also been reported to exert antimicrobial effects (Kolmas et al., 2015). The copolymer INU-bPEI-PLA was introduced to modulate the intrinsic hydrophobicity of PCL, improve blend processability through its amphiphilic nature, and provide cationic PEI domains known to disrupt bacterial membranes (Gibney et al., 2012). We hypothesized that the combined presence of Mn^{2+} ions and PEI moieties could produce a synergistic antibacterial effect while preserving the regenerative functionality of the composite.

Blends with varying compositions were prepared by solvent casting to investigate both the individual and combined effects of Mn-HA and INU-bPEI-PLA on PCL processability and resistance to microbial colonization by selected nosocomial strains. Morphological (SEM), dimensional, and thermal (DSC) analyses confirmed the successful fabrication of homogeneous composite disks suitable for preliminary antibacterial screening.

Overall, this study establishes a rational basis for selecting optimized formulations for extrusion-based 3D printing and for the subsequent development of infection-resilient scaffolds for bone tissue regeneration.

B06. Isolation and Biological Characterization of Specific Bacteriophages Against *Klebsiella pneumoniae* and *Serratia marcescens* from Hospital Wastewater and The Panama WWTP

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In response to the critical rise in multidrug-resistant (MDR) bacteria, the LAMEXA-LAMA laboratory at the University of Panama has conducted research focused on the search for bacteriophages as a therapeutic and sanitary alternative. This study involved the isolation and biological characterization of lytic phages specific to *Klebsiella pneumoniae* and *Serratia marcescens*, utilizing hospital and urban wastewater matrices from the Dr. Arnulfo Arias Madrid Hospital Complex, the Cinta Costera pumping station, and the Panama City Wastewater Treatment Plant (WWTP). The bacterial strains used were previously identified via the Vitek-2 system and endpoint PCR. As a result, 12 phages (7 for *K. pneumoniae* and 5 for *S. marcescens*) were isolated and purified using the double-layer agar technique, with subsequent tests conducted using stock concentrations of 10^7 PFU/mL. Phenotypic evaluation demonstrated that 57% of the *K. pneumoniae* phages and 20% of the *S. marcescens* isolates exhibit effective lytic activity against clinical and environmental strains with confirmed MDR profiles. Furthermore, candidates with exceptional physicochemical stability were identified, maintaining viability across a pH range of 3 to 11, thermal resistance up to 60°C, and tolerance to 90% chloroform concentrations. Additionally, phages showed suppression indices of 50% at an MOI of one. These findings lay the groundwork for the genomic sequencing and bioinformatics analysis phase, aimed at validating their application in phage therapy, biological control, and water sanitation protocols. This project seeks to consolidate a robust line of research extending to other pathogens within the ESKAPE group, strengthening Panama's scientific infrastructure against modern medical threats.

B07. Assessment of the Synergistic Activity of Imipenem/Relebactam in Combination with Meropenem and Tigecycline Against *Burkholderia cepacia* Complex Isolates

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Objectives:
The *Burkholderia cepacia* complex (BCC) comprises significant opportunistic pathogens frequently associated with healthcare-related infections and high levels of multidrug resistance. Due to extensive intrinsic and acquired resistance across multiple antimicrobial classes, these infections pose a major therapeutic challenge, particularly in immunocompromised patients. This study investigated the in vitro activity of imipenem/relebactam (IMI/REL) and evaluated its synergistic and bactericidal effects in combination with meropenem (MEM) and tigecycline (TIG) against clinical BCC isolates.

Methods:
A total of 20 non-epidemiologically related clinical BCC isolates were included. Minimum inhibitory concentrations (MICs) were determined by broth microdilution in accordance with CLSI (2023) guidelines, with *Pseudomonas aeruginosa* ATCC 27853 as the quality control strain. Synergistic potential and bactericidal activity of IMI/REL-based combinations were assessed using time-kill assays. Bacterial counts (CFU/mL) were measured over 24 hours, and log reductions were calculated relative to the initial inoculum.

Results:
IMI/REL showed 80% susceptibility against the tested BCC isolates. Based on MIC_{50} and MIC_{90} values, the activity profiles were as follows: IMI/REL (1/4 and 128/4 mg/L), MEM (4 and 8 mg/L), and TIG (2 and 4 mg/L). Time-kill analyses revealed that the IMI/REL–MEM combination produced the most pronounced synergistic effect, achieving a ≥3-log10 reduction in bacterial counts compared with the initial inoculum within 24 hours. No antagonistic interactions were detected among the tested combinations.

Conclusion:
Our findings indicate that IMI/REL has significant in vitro activity against clinical BCC isolates. The marked synergy and potent bactericidal effect observed with the IMI/REL–MEM combination highlight its potential as a promising therapeutic alternative for treating severe infections in immunocompromised patients caused by this challenging multidrug-resistant pathogen.

B08. New insights into peptide Docking and Molecular Dynamics for β-lactamase inhibitor discovery

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Since the clinical introduction of antibiotics, antimicrobial resistance (AMR) has emerged as a global challenge. AMR has been recognized as one of the leading causes of mortality worldwide, impacting human, animal, and environmental health, thereby necessitating an integrated One Health approach. In this context, β-lactamases, enzymes capable of hydrolyzing β-lactam antibiotics, stand as the most significant mechanism of AMR. Consequently, the use and search for β-lactamase inhibitors have become an essential strategy to preserve the efficacy of these antibiotics. Following the analysis of the β-lactamase TEM-1 (PDB ID: 1ERM) active site's chemical features, 34 five-residue peptides were formulated. Peptide construction was performed using the Discovery Studio Visualizer and was subsequently optimized using Foldit Standalone. Docking was carried out with the GOLD software, using the GoldScore function. To date, the three best performing peptides (Peptides 32, 33 and 34) were selected for Molecular Dynamics (MD) studies, which were performed using the Desmond program. Peptide 32 established hydrogen bonds with the conserved residues essential for catalysis (S70, S130, K73, E166, N170). Peptide 34 formed hydrogen bonds with the residues (S130, E166, N170). Furthermore, Peptide 33 exhibited hydrogen bonds with (S130, K73, E166, N170). Considering the MD studies, it was observed that two of the three peptides (Peptides 32 and 34) demonstrated stability in the active site, remaining bound throughout the simulation, which corroborates the prediction of a probable inhibition. Conversely, Peptide 33 did not maintain stable interactions and failed to remain in the active site. Specific analyses of the interactions observed during the simulation time are currently being conducted. Based on these results, these peptides, which are promising as b-lactamase inhibitors, will subsequently be synthesized for minimum inhibitory concentration (MIC) assays to confirm *in vitro* the results obtained *in silico*. Moving forward, the peptides will be investigated against other β-lactamases such as KPC and CTX-M.

B09. Cefiderocol And Mortality Outcomes in Carbapenem Resistant *Acinetobacter baumannii* Infections, A Narrative Evidence Review

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Introduction: Carbapenem-resistant *Acinetobacter baumannii* represents a global health problem due to its limited therapeutic options and high associated mortality. Cefiderocol, a novel siderophore cephalosporin derivated has emerged as a potential treatment for infections caused by multidrug resistant Gram-negative bacteria as *Acinetobacter baumannii*, however, its microbiological activity, clinical performance, and resistance patterns remain heterogeneous across different studies.

Objective: To synthesize and critically evaluate the available scientific evidence regarding the activity of cefiderocol against *Acinetobacter baumannii*, with particular emphasis on susceptibility profiles and clinical outcomes, including mortality.

Material and methods: A structured literature search was conducted in PubMed, Scopus and Cochrane data bases between the 2018 and 2025 period, where the primary search terms included "*Acinetobacter baumannii*" and "cefiderocol," both as MeSH terms. After excluding non-relevant bacterial species, 61 articles were retrieved, of those, 18 studies were excluded for not meeting the predefined inclusion criteria. Consequently, 43 studies were included in the final narrative synthesis and qualitative analysis.

Results: The integrated evidence demonstrates that cefiderocol exhibits relevant *in vitro* activity against *Acinetobacter baumannii*. *In vivo* studies indicate that cefiderocol retains bactericidal activity against susceptible isolates and that combination therapy, particularly with ampicillin/sulbactam, may enhance antimicrobial efficacy. Clinical and observational evidence evaluating mortality was limited. The available observational study reported mortality rates comparable to those previously described for carbapenem-resistant *Acinetobacter baumannii* infections, with no statistically significant difference between cefiderocol monotherapy and combination therapy.

Conclusions: Cefiderocol demonstrates consistent *in vitro* and *in vivo* activity against *Acinetobacter baumannii*, including carbapenem-resistant and colistin-resistant isolates, however, clinical evidence evaluating mortality outcomes remains limited. Therefore, while cefiderocol represents a promising option for the treatment of *Acinetobacter baumannii* infections, its impact on mortality cannot be definitively established based on current evidence, highlighting the need for further clinical studies.

B10. In Situ Mineralization of Alginate Hydrogels with Nisin: A Bioactive Material for Bone Applications

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The rise of antibiotic-resistant pathogens in osteoarticular infections underscores the need for alternative antimicrobial strategies acting locally within bone tissue. Antimicrobial peptides (AMPs) such as nisin offer broad-spectrum activity and low resistance potential, yet their clinical use is limited by rapid degradation and systemic clearance in vivo. To address this limitation, we developed alginate hydrogels capable of encapsulating and protecting nisin while enabling localized antimicrobial action. These hydrogels exhibited clear antibacterial activity against *Staphylococcus aureus* and *S. epidermidis* in a 3D agar diffusion assay, generating inhibition halos of 1.84 ± 0.34 mm in width. They also achieved complete eradication of planktonic cultures, corresponding to a ≥5-log reduction in CFU.

To enhance the bone related properties of the system, the hydrogels were mineralized using complementary strategies: (i) encapsulation of preformed calcium phosphate (CaP) powders (hydroxyapatite and β-TCP), and (ii) a novel in situ alginate mineralization method designed to improve the stability and homogeneity of crosslinking and CaP distribution throughout the hydrogel. Notably, hydrogels prepared with our novel method showed enhanced storage stability, remaining active for at least two additional weeks compared to standard preparations. Varying the duration of in situ mineralization enabled the formation of distinct CaP phases, such as hydroxyapatite and brushite, as confirmed by XRD. Importantly, CaP functionalization did not compromise antimicrobial performance, with average halo widths of 1.71 ± 0.30 mm. Preliminary analyses indicate that mineralization increases the mechanical robustness of the hydrogels, with further mechanical, rheological, and stability studies underway. These characterizations will guide the selection of the final application format (injectable, mouldable, or scaffold-like). Ongoing work also focuses on assessing bioactivity through ion release profiling and apatite formation in SBF.
Overall, this work presents a promising antimicrobial and bioactive hydrogel platform that integrates AMP delivery with CaP mineralization, offering potential for localized infection control and bone regeneration in osteoarticular applications.

B11. Study of the Behavior of the Pair *Klebsiella pneumoniae* | Carbapenems in Several European Counties by Clustering Health Data Time Series with the Generalized Affinity Coefficient

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Antimicrobial resistance (AMR) constitutes a major public health and economic burden in contemporary society. The aim of this work is to identify patterns and trends associated with the resistance behavior of *Klebsiella pneumoniae* to carbapenems across several European countries in order to better understand its dynamics and public health implications, since it's a pair considered by the WHO as a critical priority.

Variables: The dataset resistance values of *K.pneumoniae*|Carbapenems for several countries from 2005 to 2021(data available up to 2023) were obtained from the public website of ECDC.

Methods: Agglomerative Hierarchical Cluster Analysis (HCA) was based on the generalized Affinity coefficient applied to the estimated ARIMA models.

Results: The dendrogram at the cut-off presented four clusters: the first cluster was Slovenia, Luxembourg, Italy, and Estonia; the second cluster was Romania, Malta, Greece, Portugal, and Spain; the third cluster was Denmark, France, and Czechia; and finally, the fourth cluster was Lithuania, Cyprus, Finland, Austria, Belgium, and Slovakia. Although the resistance values show a more visible increasing trend over the years in Clusters II and III, the increase is more pronounced. Data were standardized, and the slopes of the lines obtained between the years 2012 and 2020 were calculated for Clusters II and III. For Clusters I and IV, this was not possible due to the irregularity of the curves. The slopes are higher in the countries belonging to Cluster II. Specifically, the slopes for Cluster II's countries are m=0.29, m=1.5, m=0.06, m=0.11, and m=0.26 for Portugal, Spain, Greece, Malta, and Romania, respectively, whereas for Cluster III's countries, the slopes are m=0.02, m=0,08 and m=0.001 for France, Czechia, and Denmark, respectively. It is relevant that within Cluster II, Portugal and Spain show a steeper increase, with Spain showing the most pronounced growth.

Conclusions: The application of data analysis, using HCA based on the generalized Affinity coefficient, allows the evaluation of patterns among different regions and gives the possibility to identify alarming groups that need urgent intervention

B12. *Halobacteriovorax* to Kill Multi-Drug-Resistant *Escherichia coli* and *Salmonella*

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Background/Objectives: Due to the rising problem of antimicrobial resistance, there is increasing attention in the scientific community towards alternative approaches to combat Antimicrobial-Resistant (AMR) pathogens that do not involve the use of antibiotics. In this regard, the European Medicines Agency (EMA) and the European Food Safety Authority (EFSA) have promoted experimentation with predatory bacteria to fight antibiotic resistance. In this work we isolated a strain of *Halobacteriovorax* from an estuarine aquatic environment using a CTX-M-producing *E. coli* strain as prey and characterized it with respect to optimal physico-chemical parameters for growth and predation. Furthermore, we studied its predatory capacity against other *E. coli* strains and Multi-Drug-Resistant (MDR) *Salmonella*. Finally, we conducted challenge experiments to evaluate the growth of predator and prey over time.

Methods: The *Halobacteriovorax* strain, designated HE7, was identified by 16S rRNA analysis. To isolate *Halobacteriovorax* and to evaluate its predatory ability towards different preys, the double-layer agar plating technique was applied.

Results: HE7 showed in vitro predatory activity against all MDR strains of *E. coli* and *Salmonella* tested. In the 10^7 predator/10^3 prey and 10^7 predator/10^7 prey challenges, HE7 after 6 h achieved the total killing and a reduction of about 6 logs in the prey, respectively, maintaining this effect for up to 24 h.

Conclusions: The results of this study highlight that HE7, but more generally *Halobacteriovorax*, could find application both alone and in an integrated context of antimicrobial strategies as an alternative to antibiotics.

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B13. Exploring Antimicrobial Potential of Mytilus Genomic Resources

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The global healthcare community faces escalating threats from antimicrobial resistance (AMR); therefore, the need for novel, non-traditional antimicrobials has become increasingly urgent. Antimicrobial peptides (AMPs), naturally produced by a wide array of organisms, including marine invertebrates, offer a promising alternative to conventional antibiotics. AMPs typically act by disrupting microbial membranes, a mechanism less prone to resistance development. Mussels, being sessile filter feeders in pathogen-rich marine environments, have evolved expansive and diverse innate immune systems. The genomic diversity and adaptive resilience of Mytilus spp. remain underexploited resources for drug discovery, particularly in the search for new AMPs with clinical and ecological relevance.

The central objective of the project is to identify, functionally validate, and characterize novel AMPs from Mytilus genomes. The research plan integrates in silico genomic screening with experimental in vitro validation. Initially, genome assemblies and transcriptomic datasets from multiple Mytilus species will be analyzed to identify gene families coding for AMP-like sequences. The bioinformatics strategy includes sequence motif detection, gene clustering, evolutionary analysis of presence/absence variation, and structural modeling of predicted peptides. These tasks will be supported by national HPC resources provided through the PLGrid infrastructure, which allows high-throughput data processing, molecular simulations, and structural predictions. Experimentally, we will establish short-term primary cultures from Mytilus hemocytes and digestive gland cells. These cultures will serve as models to assess immune gene expression and AMP induction under controlled stimulation. Culture viability and responsiveness will be monitored using fluorescein diacetate (FDA) assays and quantitative PCR. Peptides derived from highly expressed or computationally prioritized genes will be purified either from conditioned media or synthesized chemically when necessary. Functional testing will involve comprehensive antimicrobial profiling against a library of multidrug-resistant strains.

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B14. Dual Approach to Reduce Toxicity in Polymyxins and Regain Activity Against Colistin-Resistant Strains

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Antibacterial resistance is rising globally, with particular concern involving multidrug-resistant Gram-negative pathogens listed in the WHO Global Priority List (2024), i. e. carbapenem-resistant and third-generation cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* and also carbapenem-resistant *Pseudomonas aeruginosa*. In this study, we present a dual strategy to reduce polymyxin B and colistin toxicity while maintaining efficacy in the murine model of infection. We used a disulfide-based chemical modification (soft-drug approach) alongside a combination therapy with a clinically approved compound. We will present *in vitro* microbiological testing (MIC against MDR gram negative bacteria including colistin-resistant strains such as *K. pneumoniae* ST147), *in vivo* pharmacokinetic data, acute toxicity and safety evaluation (nephrotoxicity and biomarkers KIM-1, clusterin, NGAL, THF-α) and efficacy in clinically relevant murine infection sepsis models. Taken together, the combination approach increased the tolerated dose of polymyxins by up to fivefold in mice, from 20 to 100 mg/kg subcutaneously and from 4 to 20 mg/kg intravenously, while maintaining antibacterial efficacy.
(https://jpiamr.eu/projects/muryxin/)

B15. Searching for Novel Adjuvants of β-lactam Antibiotics to Contrast Multidrug Resistant Gram-Negative Bacteria

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β-lactams represent cornerstone antibiotics, but resistance towards them is widespread [1]. Of particular concern are β-lactam-resistant Gram-negative bacteria, for which alternative therapeutic options are extremely limited. To address this unmet medical need, we developed a natural product screening platform for searching antibiotic adjuvants to be used in combination with β-lactams against multidrug-resistant Gram-negative (MDR-GN) bacteria. To this end, we first selected five clinically-relevant models of MDR-GN characterized by β-lactam resistance (with MICs of ceftriaxone, meropenem, and/or ceftazidime–avibactam in the range 8-1024 μg/ml) and we identified the β-lactamases acting as resistance determinants (namely, CTX-M, KPC-3, KPC-31, VIM-1, and VIM-2). Then, we performed the high-throughput biological activity-guided screening of a microbial library comprising 39,000 crude extracts mainly obtained from actinomycetes' and filamentous fungi's culture broths. The screening assay was developed to select putative HITs with no or negligible direct antimicrobial activity, but capable of counteracting resistance mechanisms by inhibiting β-lactamase activity. Notably, >200 HITs capable of restoring the activity of β-lactams against the selected resistant isolates, even when the antibiotics were used at 1/4 or 1/8 the MIC value, were identified. The candidates were further subjected to activity-guided purification, resulting in the isolation of several compounds with the desired adjuvant activity, which underwent chemical and biological characterization for dereplication and novelty assessment. Preliminary findings suggested that the observed bioactivities were mostly associated to metal chelating agents. As further perspectives, an in-depth characterization of these compounds is being conducted, to better elucidate their mode of action through in vitro and in silico studies, to test their efficacy in conjunction with β-lactams against a broader panel of MDR-GN clinical isolates, and to assess their cytotoxicity on various eukaryotic cell models, ultimately paving the way for their possible preclinical development and prospective therapeutic application.

[1] GBD 2021 Antimicrobial Resistance Collaborators. (2024). *The Lancet*. 404(10459):1199-1226.

B16. Evaluation Of Antibiotic Use in Patients Admitted to the Intensive Care Unit (ICU) with Pneumonia-Sepsis: Retrospective Observational Before-After Study

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Early and adequate empiric antibiotic therapy is essential in the treatment of pneumonia–sepsis, influencing clinical outcomes. This retrospective before–after study aimed to appraise the impact of local ASP (written guidelines and antibiotic restriction) on antibiotic (AB) use and clinical outcomes in patients requiring intensive care due to pneumonia–sepsis. This single-center retrospective observational study was conducted in the ICU of a pulmonology department in Hungary. Data collection for the pre-intervention period occurred from January 2018 to May 2022, while that for the ASP period was from June 2022 to March 2024. The patients admitted to the ICU with pneumonia–sepsis were mainly men (58/101, 57.4% and 84/128, 65.6%); the need for ICU increased with age, and most of the patients belonged to 65+ age group in both study phases (68.3% and 58.6%). The majority of the patients had four or more comorbidities (57.4% and 40.6%). In-hospital mortality was relatively high (42.6% and 41.4%), with most of the patients already losing their lives in the ICU (33/43, 76.7% and 37/53, 69.8%). A significant increase in guideline-adherent agent selection (34.5%) and the use of combination therapy (35.0%) was observed, while the use of fluoroquinolones decreased significantly (-31.1%). In the after period, a significant decrease in the number of patients using restricted ABs (-53.3%) was observed. In one-third of these cases (10/34, 29.4% and 16/40, 40%), 2–4 MDR pathogens were detected simultaneously, resulting in a significant increase in direct costs (10.5%). The use of inappropriate AB therapy was relatively low in the presence of MDRs in both phases (5.9% and 15%). In the ASP period, guideline adherence was associated with slightly better clinical outcomes (30-day mortality: -0.8% and LOS: -22.6%). Interrupted time series analysis (ITS) indicates a decrease in the trend of AB use in the ICU (from 0.16 /30 days to -0.22 /30 days). ASP implementation in the ICU resulted in a significant improvement in the appropriate use of ABs, and guideline adherence led to slightly better clinical outcomes. ASP may hold promise in improving AMR with a sustained long-term effect.

B17. Unravelling bla_{IMP} Carbapenemase Gene Genetic Context in Dairy-Cattle and Environmental Metagenomes

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INTRODUCTION: Carbapenemase-producing organisms (CPOs) pose a major global health threat. According to EARS-Net, Greece has one of Europe's highest CPO-incidences in healthcare-associated infections, endemic for KPC, NDM, VIM, and OXA-48-like-carrying CPOs, but not for IMP. This study was conducted on metagenomes recovered from intensive dairy farms in Greece and harbored *bla*$_{IMP}$-determinants to explore their genetic context and dynamics underlying *bla*$_{IMP}$-dissemination, adopting a One Health-related approach.

METHODS: Short reads and contigs were comparatively analyzed using BLASTn, MEGA, and Easyfig software; assembled with MEGAHIT; and mapped to references (MZ816709.1, AJ550807.1) using Burrows-Wheeler-Aligner and SAMtools.

RESULTS: Analyses revealed that *bla*$_{IMP}$-associated reads were identical or highly similar to *bla*$_{IMP-13}$. Numerous reads and contigs covered the entire length of a Tn402-like transposon, including transposase (*tnpA/R/M, tniA/B*) and mercury-resistance (*merRTPCADE*) genes and a class-1 integron harboring *bla*$_{IMP-13}$ variant, *aac(6')*, *aadA1*, *qacEdelta1,* and *sul1*, but lacking *qacG2* genes.

DISCUSSION/CONCLUSIONS: Tn402-like transposons as carriers of class-1 integrons harboring *bla*$_{IMP-13}$ variants have mainly been restricted to isolates from human clinical settings, promoted by the intensive use of carbapenems in these environments. Since carbapenems are banned from use in food-producing animals, it is surprising to note this highly mobile genetic element to be recovered from dairy cattle feces and environments. This suggests that any associated horizontal transfer could introduce *bla*$_{IMP-13}$ variants into novel hosts, with the human–agricultural interface being a critical hotspot for spillovers. Given that *bla*$_{IMP}$ has not yet been reported in human clinical settings in Greece, moving personnel from areas with high *bla*$_{IMP-13}$-carrying CPO incidence could be a potential source, creating secondary reservoirs and forming a cycle that changes traditional epidemiological boundaries. This is highly concerning, as widely used veterinary antibiotics could provide co-selection pressure, favoring the spread of *bla*$_{IMP-13}$-variant-carrying CPOs. Our findings highlight the need for proactive metagenomic surveillance and strengthened monitoring strategies.

B18. Advance in Developing Antimicrobial Peptides with Systemic Efficacy

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Antibiotic resistance is a global problem that requires different strategies, including new types of antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) and drug-resistant *Pseudomonas aeruginosa* are involved in skin infections, ventilator-associated pneumonia, and bacteremia. This poster reports an improved peptide antibiotic for treating such challenging infections. We designed a minimal peptide verine previously by combining LL-37 membrane-targeting elements with our peptide design concept for systemic use (PNAS 2020;117:19446-19454). Here we advanced verine based on the findings from the antimicrobial peptide database version 6 (APD6; Nucleic Acids Res. 2026;54:D363-D374). Aksarbin, the improved peptide, showed a favorable in vitro cytotoxicity profile to six cell lines comparable to daptomycin. In vivo, the peptide exhibited a single maximum tolerated dose exceeding 100 mg/kg and demonstrated no toxicity to mice following one week of intravenous injection at the treatment dose of 10 mg/kg. Aksarbin was potent against gentamicin, piperacillin, ceftazidime, colistin-resistant clinical strains, New Delhi Metallo-β-lactamase (NAM) strains, gram-positive MRSA, and extended-spectrum beta-lactamase (ESBL) gram-negative *Escherichia coli, P. aeruginosa*, and *Klebsiella pneumoniae* strains. It also showed antibiofilm capability and suppressed bacterial virulence factors based on transcriptomic and proteomic studies. In addition, bacteria did not develop resistance to aksarbin after multiple passages experiment. Significantly, aksarbin demonstrated topical efficacy in *S. aureus* USA300 infected biofilm wounds, and systemic efficacy in *P. aeruginosa*-infected bacteremia and overnight infected pneumonia murine models. Combined, these results underscore the advance in designing antimicrobial peptides with systemic efficacy and the therapeutic potential of aksarbin in combating drug-resistant pathogens, especially difficult-to-kill gram-negative bacteria.

B19. Docking-based Screening and Interaction Analysis of β-lactamase Inhibitors Targeting TEM-1, KPC-2 and BcII

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This study aims to identify and characterize, through *in silico* approaches, potential inhibitors of serine- and metallo-β-lactamases, enzymes directly associated with antimicrobial resistance (AMR). The ChEMBL database was screened to select molecules with reported bioactivity against TEM-1 (PDB ID: 1ERM) and KPC-2 (PDB ID: 6D15), both class A enzymes, and BcII (PDB ID: 2M5D), a class B enzyme. Based on IC_{50} values, 200 molecules were selected for TEM-1, 89 for KPC-2, and 100 for BcII. The protonation states of the selected compounds at pH 7.4 were determined using MarvinSketch. Three-dimensional structures were built in Discovery Studio Visualizer and optimized using the semiempirical PM7 method implemented in MOPAC. Protocol validation was carried out by redocking the co-crystallized ligands, followed by molecular docking simulations of the selected compounds. Docking results were analyzed using dbCICA, which correlates binding affinity with interaction patterns in the enzyme active site. Post-processing of docking data enabled the identification of intermolecular interaction patterns that may assist the rational design of novel inhibitors. The most active ligands established multiple hydrogen bonds with conserved catalytic residues, including SER70, SER130, GLU166, ASN170, and LYS73. In contrast, less active compounds exhibited fewer interactions with these key residues. The dbCICA models showed limited predictive performance, with low coefficients of determination (r^2 = 0.24 for TEM-1, 0.37 for KPC-2, and 0.29 for BcII). Among the evaluated residues, GLU166 was consistently identified as relevant for both class A enzymes. Despite the modest statistical performance, the findings indicate that potent inhibitors tend to interact with essential catalytic residues, providing mechanistic insights. These interaction signatures will guide pharmacophore modeling and ZINC-based virtual screening, followed by in vitro validation to identify β-lactamase inhibitors as antibiotic adjuvants against AMR.

B20. Functional Characterization Approach to Identify Novel Antimicrobial Resistance Genes Using Cosmid Libraries from *Pseudomonas* spp.

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Bacterial infections pose a significant threat to global health, especially with the growing resistance to antibiotics. Among critical priority pathogens, *Pseudomonas* species are of particular concern due to their intrinsic resistance mechanisms and capacity to acquire novel resistance genes. Despite their clinical importance, relatively few studies have characterized antimicrobial resistance genes (ARGs) in environmental *Pseudomonas* strains, especially those encoding β-lactamases.
In this study, we analyzed a collection of 56 bacterial genomes isolated under carbapenem antibiotic selection from municipal and hospital wastewater systems across Ontario. Whole-genome sequencing revealed that 33 of these isolates belonged to 11 *Pseudomonas* species; further annotation in CARD and ResFinder databases identified the presence of known ARGs across all isolates. However, most of the carbapenem-resistant isolates did not show observable β-lactamases or carbapenemase gene determinants. To investigate the nature of the antibiotic resistance, we constructed cosmid-based genomic libraries using DNA extracted from highly resistant *Pseudomonas* isolates. After confirming carbapenem resistance, the positive clones were sequenced using Oxford Nanopore Technology. Further analysis showed a group of clones with overlapping regions when mapped to the reference genome, containing several uncharacterized coding sequences. Through functional screening of these clones, subcloning and Tn5 mutagenesis, we aim to identify and characterize novel resistance genes, with particular emphasis on uncharacterized β-lactamase and carbapenemase activities. This approach not only supports the detection of ARGs that may be overlooked by sequence-based methods but also enables the characterization of their phenotypic effects and potential clinical implications of ARGs found in non-clinical settings. Furthermore, it emphasizes the importance of monitoring wastewater as a reservoir system for emerging resistance threats.

B21. Nature's Nanofactory: Leveraging Medicinal Plants as Novel Therapeutics

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With the emergence of antibiotic resistance, there is an increase in illness, death, and healthcare costs and it also poses a threat to medical procedures. Novel therapeutic agents with a multi-pronged effect are therefore urgently required so as to impede bacterial mutations that lead to antibiotic resistance strategies. Metallic nanoparticles, particularly those containing silver, have been showing promise to fulfil this role and these nanoscale powerhouses could be our answer. Green biochemical synthetic strategies coupled with traditional medicine have therefore gained popularity as it results in an eco-friendly strategy for nanoparticle synthesis and the added benefit of medicinal phytochemicals being incorporated into the nanoparticle thereby resulting in a potent antibacterial agent. This study therefore made use of the medicinal plant, *Siphonochilus aethiopicus*, which is known to contain antibiotic compounds for the synthesis of silver nanoparticles. These nanoparticles were found to have excellent broad-spectrum antibiotic activity when tested using the INT assay. When the silver nanoparticles were tested against *Acinetobacter baumannii* (ATCC 19606), concentrations of 50 µg/ml and higher resulted in more than 90% toxicity. Against *Pseudomonas aeruginosa* (ATCC 27853), more than 80% toxicity was achieved with 25 µg/ml of the silver nanoparticles and more than 90% toxicity resulted when concentrations of 50 µg/ml or higher were evaluated. These results were comparable to the positive control ciprofloxacin. The silver nanoparticles also displayed more than 90% toxicity against *Enterococcus faecalis* (ATCC 51299) at concentrations of 25 µg/ml and higher, comparable to the positive control ampicillin. Against *Staphylococcus aureus* (ATCC 43300), more than 90% toxicity was achieved at concentrations of 25 µg/ml and higher, and of note these results indicated that the silver nanoparticles performed slightly better than the positive control ciprofloxacin. These nanoparticles therefore show excellent potential to be further developed as novel antibiotics.

B22. Antibacterial Potential of Organ-Specific Extracts from *Ebenus pinnata* Aiton, Correlation with Phenolic Composition revealed by UHPLC–ESI–MS

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The increasing prevalence of antibiotic-resistant bacteria highlights the urgent need for alternative antimicrobial agents derived from natural sources. In this study, we investigated the organ-specific phytochemical composition and antibacterial potential of hydroethanolic extracts obtained from flowers, leaves, and stems of *Ebenus pinnata* Aiton, an endemic Moroccan species. Comprehensive metabolite profiling was performed using UHPLC–ESI–MS, while total phenolic (TPC), flavonoid (TFC), and proanthocyanidin (TPrC) contents were quantified spectrophotometrically. Antibacterial activity was evaluated using agar diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays against representative Gram-positive and Gram-negative bacteria.

UHPLC–ESI–MS analysis revealed pronounced organ-specific chemical signatures. Flowers were enriched in flavanols, particularly epigallocatechin, alongside flavonoids and phenolic glycosides. Leaves were dominated by glycosylated flavonoids and hydroxycinnamic acids, whereas stems accumulated diverse phenolic acids, proanthocyanidins, and triterpenoids, including ursolic acid. Quantitative assays confirmed significant inter-organ variability, with stems exhibiting the highest TPC, TFC, and TPrC values, followed by leaves and flowers. Antibacterial assays demonstrated a clear selectivity toward Gram-positive bacteria. Flower and stem extracts showed the strongest activity, particularly against *Micrococcus luteus* and *Staphylococcus aureus*, with low MIC values (2.60–5.20 mg/mL) and bactericidal effects (MBC/MIC ≤ 2.50). In contrast, Gram-negative strains were markedly less susceptible.

Antibacterial efficacy correlated more strongly with the qualitative composition of phenolic constituents, especially flavanols and proanthocyanidins, than with total phenolic abundance. These findings highlight the importance of organ-specific phytochemical allocation in shaping antimicrobial activity and position *E. pinnata*, particularly its floral and stem tissues, as a promising source of bioactive phenolics with potential relevance for the development of plant-derived antibacterial agents.

B23. Biochemical and Structural characterisation of the MurCDdl Chimera in *Chlamydia pneumoniae*

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Most bacteria contain a peptidoglycan cell wall that encapsulates the bacterial cytoplasmic membrane, maintaining cell shape and integrity. While the cell wall is a major target for antibiotics, very few target the initial steps of peptidoglycan biosynthesis that occurs in the cytoplasm. This involves a series of Mur enzymes which catalyse the formation of the important intermediate UDP-MurNAc-pentapeptide. While the Mur enzymes are usually expressed as separate proteins, in some bacteria they are synthesised as naturally occurring chimeric proteins, such as MurC-Ddl in *Chlamydial* species. Unlike most chimeric proteins, the MurC and Ddl enzymes do not catalyse sequential reactions but may be linked due to allostery between the two enzymatic domains; the Ddl domain was inhibited by UDP-MurNAc and UDP-MurNAc-Ala, a substrate and product of MurC respectively, as well as an inhibitor of MurC. The structure of MurC-Ddl was resolved to 2.8 Å and revealed the MurC inhibitor bound to the MurC domain only. Additionally, MurC and Ddl could be fused in *Chlamydia* for stability since neither of these enzymatic domains could be expressed as isolated proteins that retained activity. Ddl in particular was completely unstable and may require the MurC domain to stabilise a large α-helix protruding from the protein that occurs at the C-terminus of Ddl. The clinical targeting of Mur ligases, such as MurC, remains under-developed and therefore these enzymes could represent good targets for the development of novel antibiotics. Since MurC and Ddl share a similar catalytic mechanism, MurC-Ddl could be amenable to multi-target inhibitors and the structure of MurC-Ddl may help direct the future development of novel inhibitors targeting this chimera. Furthermore, MurC inhibitors are likely to target other Mur ligases owing to their common structure and catalytic mechanism.

B24. Structural Determinants of Systemic Antibiotic Use in Hungary, 2010 To 2022: Empirical Evidence for Policy Action

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Introduction: The consequences of antimicrobial resistance (AMR) are observed across different socio-economic strata and geographical regions; however, its key drivers and consequences are exacerbated by persisting economic and social inequalities. The AMR Quadripartite and the United Nations (UN) High-Level Political Forum have both empasized the importance of intersectoral actions addressing structual inequalities, as well as the need for sustained political commitment and policy predictability.

Methods: An ecological study was carried out based on secondary data collection, where the association between systemic (including community and hospital-based) antibiotic use (SABU) and thirty-three (N=33) indicators—corresponding to economic, developmental, political conditions, health systems status and inequality—for Hungary were assessed, over the period of 2010–2022. Data on SABU, expressed as defined daily doses per 1,000 inhabitants per day (DDD/1,000 inhabitants/day), were obtained from the European Centre for Disease Prevention and Control (ECDC) ESAC-Net database, while other indicators were sourced from the Hungarian Central Statistical Office (KSH), WHO Health for All, UN Human Development Reports, Our World in Data, and the World Bank DataBank databases, respectively. Statistical analyses (descriptive statistics, Spearman's rho [ρ]) were conducted using jamovi version 2.4.5. The study followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines.

Results: SABU showed negative correlations with the mean number of years in education (r_s=−0.688; p=0.009), number of nurses and midwives/10,000 inhabitants (r_s=−0.564; p=0.044), gross domestic product (GDP) per capita (r_s=−0.523; p=0.067), political stability index (r_s=−0.529; p=0.062), gross national product (GNP) (r_s=−0.502; p=0.081), and the Gini coefficient (r_s=−0.484; p=0.094). Conversely, positive correlations were observed between SABU and the human rights index (r_s=0.565; p=0.044) and with the proportion of the population living in poverty (r_s=0.517; p=0.070).

Conclusions: Incorporating social stratifiers and the perspectives of intersectional is warranted in AMR-related data collection (e.g., surveillance), policy design and mitigation of AMR-related inequalities.

B25. Antimicrobial Resistance-Related Knowledge and Practices Among Biology and Pharmacy Students: A Questionnaire-Based, Single-Centre, Cross-Sectional Study Applying the Special Eurobarometer 478 Methodology

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Introduction: Inappropriate use of antibiotics is one of the main drivers of antimicrobial resistance (AMR). The „*Competency Framework for Education and Training on AMR for Health Workers*" (WHO, 2018) defines the knowledge, attitudes, and practices required throughout the various domains of healthcare—including direct patient care, pharmaceutical services, laboratory diagnostics, and health management—to effectively reduce AMR-related burden.

Methods: A quantitative, single-center cross-sectional study was performed with purposive sampling among undergraduate biology and pharmacy students between 01/03/2021 and 01/02/2022. Data collection on AMR-related knowledge and practices was carried out using a 70-item, self-administered, online questionnaire based on the methodology of the Special Eurobarometer (EBM) 478 survey (European Commission, 2018). In addition, participants' ability to recognize AMR-related terms and to identify pharmaceuticals (as antibiotic vs. non-antibiotic) was also assessed. Statistical analyses (descriptive statistics, Welch's t-tests, and Pearson correlation) were performed using IBM SPSS 22.0. The study followed the Checklist for Reporting Results of Internet E-Surveys (CHERRIES) guidelines.

Results: Among participants (N=388), 20.4% reported antibiotic use in the past 12 months; 90.7% obtained antibiotics via medical prescription, of which only 18.8% were preceded by sampling or microbiological tests. Frequently reported indications for antibiotic use were sore throat (37.9%), cough (21.9%), and fever (21.6%). No relevant associations were identified between AMR-related knowledge and gender, academic performance, or type of study ($p > 0.05$). Strong, positive correlations were shown among AMR-related knowledge scores, the number of correctly recognized AMR-related terms, and the number of correctly identified pharmaceutical agents (r=0.644 and r=0.518, respectively; $p<0.001$).

Conclusions: Graduate biologists and pharmacists possess specialized competencies that are essential for mitigating AMR. Similarly to recent, population-based EBM surveys, our sample frequently reported antibiotic use for indications suggestive of antibiotic consumption in inappropriate indications. Our findings highlight the need for continuous evaluation and targeted strengthening of AMR-related content within undergraduate curricula.

B26. Physicians' Knowledge, Attitudes, and Practices Related to the Management of *Helicobacter pylori*-Induced Gastric Ulcers: A Questionnaire-Based, Cross-Sectional Study in Pakistan

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Introduction: In South Asia, the prevalence of *Helicobacter pylori* (*H. pylori*) infections may be as high as 60-80%, constituting a notable public health issue, with eradication strategies critical in reducing the *H. pylori*-associated disease burden. The aim of our study was to assess the knowledge, attitude and practices (KAP) regarding the screening, treatment, and follow-up of *H. pylori*-induced gastric ulcers among physicians in Lahore, Pakistan.

Methods: A self-administered, questionnaire-based cross-sectional study—including the development and validity assessment of a 25-item questionnaire—was carried out in two tertiary-care hospitals between January and May 2024. Statistical analyses (descriptive statistics, χ^2-tests, binary logistic regression; 95% confidence interval [95%CI]) were carried out using SPSS 27.0. The study followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines.

Results: Among *N*=385 participants, 57.9% were male, 60.0% were aged between 25 and 34 years, 59.5% worked in a public hospital, and 55.3% had <5 years of working experience. A total of 32.9% had noted medical journals, while 27.0% reported online educational materials as their key sources of evidence-based information. Although 91.2% and 87.3% of physicians had good knowledge and attitude levels (≥50% score) pertaining to *H. pylori*-associated gastric ulcers, respectively, only 49.6% correctly identified *H. pylori* as being contagious. Participants aged 25–34 years (aOR: 0.217 [95% CI: 0.08-0.589]), who have <5 years of working experience (aOR: 0.328 [95% CI: 0.136-0.790]) and those working in public hospitals (aOR: 0.130 [95% CI: 0.048-0.352]) were less likely to show poor attitudes. A total of 76.5% made it routine to discuss the risk factors of *H. pylori*-induced ulcers with their patients, while 67.4% highlighted the importance of follow-up testing to confirm the eradication of *H. pylori*.

Conclusions: Inconsistent and empirical treatment approaches, and lack of routine screening and follow-up practices may further compromise eradication efforts, and contribute to the development of multidrug-resistance in *H. pylori*.

B27. SIOOT oxygen-ozone therapy against multidrug resistant bacteria. Perspectives and future remarks

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Introduction
Antibiotic resistance is one of the most pressing global health threats, diminishing the effectiveness of standard antimicrobial therapies and contributing to increased morbidity, mortality, and healthcare costs. In response to this challenge, ozone therapy has emerged as a potential adjunctive treatment against multidrug-resistant (MDR) bacterial infections. Notably, protocols endorsed by the Italian Scientific Society of Oxygen-Ozone Therapy (SIOOT) and clinical insights from Prof. Marianno Franzini, have advanced understanding of ozone dual mechanisms of action in infection control.

Methods
This review synthesizes data from experimental studies, modelling analyses, and clinical reports on the use of medical ozone in MDR infections. Mechanistic insights focus on two principal modes: (1) direct bactericidal activity of ozone and its reactive species through oxidative damage to microbial membranes and thiol groups, and (2) systemic immunomodulation via low-dose ozone exposure. The latter is mediated by secondary messengers such as 4-hydroxynonenal, which activate Nrf2 signaling, regulate inflammasomes, promote macrophage activity, and enhance mitochondrial function.

Results
Evidence indicates that ozone exerts rapid, broad-spectrum bactericidal effects largely independent of classical resistance mechanisms. When administered systemically within a hormetic dose range, ozone enhances host innate immunity through redox-sensitive signaling pathways and promotes tissue-level resilience. Modelling studies suggest synergistic effects when ozone is combined with antibiotics, accelerating pathogen clearance compared to antibiotic monotherapy. Clinically, ozone has demonstrated efficacy as an adjunct in treating chronic wounds and necrotizing infections caused by MDR pathogens.

Conclusions
Ozone therapy, when precisely dosed and integrated into standardized medical protocols, offers a compelling adjunctive approach in the management of antibiotic-resistant infections. Rather than replacing antibiotics, ozone augments their effectiveness and may reduce the selective pressure driving resistance. Future integration of ozone into antimicrobial stewardship programs could support more sustainable, system-based infection control strategies aligned with global health imperatives.

B28. Phage-Loaded Biomimetic Apatite Powder and Biofilms

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Bacterial biofilms represent a major challenge in the treatment of chronic infections due to their high tolerance to conventional antibiotics, notably seen in bone and joint infections. Bacteriophage therapy has re-emerged as a promising alternative or complementary strategy to address these limitations. In our lab, we investigate the use of bacteriophages encapsulated within a calcium phosphate-based delivery system known as biomimetic apatite powder, designed to mimic the mineral phase of bone tissue and ensure localized antimicrobial activity.

Phages specifically targeting *Staphylococcus aureus* and *Escherichia coli* were incorporated into this biomimetic apatite material using a precipitation process that preserves phage viability. The antibacterial efficacy of this system was evaluated against both planctonic infection and mature biofilms formed by abovementioned bacteriae. Our results demonstrate that phage-loaded biomimetic apatite significantly reduces viable bacterial counts and biofilm activity, highlighting the capacity of this encapsulation system to deliver active phages in a sustained and localized manner. Planctonic culture of *E. coli* and *S. aureus* were completely eradicated (0 CFU, 7 log reduction) after 2 hours of incubation with the phage loaded material. Biofilm culture of S.aureus were almost completely eradicated (~6,4 CFU, 5 log reduction) after 5 days of incubation with the lyophiliosed phage-loaded material but not with the lyophilised version (~2,8.10^3 CFU, 3 log reduction).

Furthermore, we explored the synergistic effects between phage therapy and conventional antibiotics. Combined treatments resulted in enhanced biofilm disruption and bacterial eradication compared to phages or antibiotics alone, suggesting a strong synergistic interaction. This combined approach may help overcome antibiotic tolerance associated with biofilms while reducing the required antibiotic doses as shown in other studies.

Overall, our findings support the potential of biomimetic apatite powders as an effective delivery platform for bacteriophages and as a promising strategy for the treatment of biofilm-associated infections, particularly in the context of bone and joint diseases.

B29. Extending The Potency And Lifespan Of Antibiotics: Inhibitors Of Gram-Negative Bacterial Efflux Pumps

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The Global Antibiotic Research & Development Partnership (GARDP) accelerates the development and access of treatments for drug-resistant infections. Together with public, private, and non-profit partners, GARDP works in partnership to develop new antibiotics and expand access to them. Through our Discovery & Exploratory Research (DER) programme, GARDP focuses on the search for novel molecules and new bacterial targets against WHO-critical Gram-negative bacterial pathogens. This work contributes to the global antibiotic R&D pipeline, aiming to create a steady stream of promising new candidate drugs. One research area of the DER programme focuses on the discovery of potentiating compounds that can restore the activity of clinical antibiotics lost to resistance, such as efflux inhibitors.

Many antibiotics are substrates of bacterial efflux pumps, and modifications to the structure or overexpression of efflux pumps are an important resistance mechanism used by many multidrug-resistant bacteria. Therefore, chemical inhibition of bacterial efflux to revitalize existing antibiotics is considered a promising approach for antimicrobial chemotherapy. We recently provided an overview of clinically relevant multidrug resistance efflux pumps in Gram-negative bacteria and further described over 50 efflux inhibitors that target such systems. Following our medicinal chemistry and microbiology review, we selected promising efflux inhibitors for benchmarking, using a *K. pneumoniae ΔramR* mutant with decreased susceptibility to antibiotics effluxed by the AcrAB-TolC system. The results will determine if these compounds are viable starting points for hit-to-lead projects focused on efflux inhibition in Gram-negative bacteria.

B30. Design and Evaluation of Metallacarborane-Peptide Conjugates as Novel Antimicrobial Agents

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Antimicrobial resistance (AMR) poses a significant global health threat, ranking among the top ten threats to humanity according to the World Health Organization [1]. The rise of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) pathogens is linked to their increasing insensitivity to traditional antibiotics [2]. Antimicrobial peptides (AMPs) offer a promising solution to combat drug-resistant infections [3]. These peptides, which possess cationic and hydrophobic regions, can disrupt bacterial cell membranes, leading to bacterial death [4]. However, challenges such as instability, high costs, and toxicity to normal cells must be addressed. Metallacarboranes are stable compounds consisting of one or more boron hydride clusters coordinating a metal cation. They possess unique structures and properties that make them a promising platform for the development of new antimicrobial agents [5].

In our studies, we synthesized a series of metallacarborane–ultrashort cationic peptide conjugates and comprehensively characterized their physicochemical properties. Their antimicrobial activity was evaluated by determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against representative Gram-positive and Gram-negative bacteria, demonstrating broad-spectrum and predominantly bactericidal effects at low micromolar levels. In parallel, cytotoxicity and hemolysis assays were performed, enabling calculation of selectivity indices that relate MIC/MBC values to toxicity thresholds and thus substantiate the favorable therapeutic window of these conjugates.

B31. Computational Evaluation of Mel4 and Lactoferricin Interactions with Adenovirus and Norovirus Capsids

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Human adenovirus and norovirus are environmentally stable, non-enveloped viruses that remain major causes of infections worldwide. Despite their clinical significance, effective antiviral therapeutics targeting these pathogens remain limited. Previous *in vitro* studies demonstrated that the antimicrobial peptides Mel4 and lactoferricin (LFc) possess antiviral activity against adenovirus type 5 (HAdV-5) and murine norovirus type 1 (MNV-1) and do not cause toxicity at their antiviral concentrations to the host mammalian cells. The ID_{50} of Mel4 was lower than that of LFc. However, although some data indicated that the mode of action was directed at the capsids of these viruses, the molecular basis of the antiviral activity was not fully elucidated. In this study, *in silico* approaches were employed, such as HDOCK docking and molecular dynamics (MD) simulations, to understand how Mel4 and LFc interact with key capsid proteins. Structural models of Mel4 and LFc were generated using AlphaFold3, and their physicochemical properties were characterised. Docking analyses against the HAdV-5 hexon, penton base, and fibre knob proteins, as well as the MNV-1 VP1 capsid protein, revealed consistently stronger binding affinities for Mel4 compared with LFc. MD simulations (100 ns) further demonstrated that Mel4–capsid complexes exhibited lower root mean square deviation values, reduced interfacial flexibility, and greater structural compactness relative to LFc complexes, indicating stronger and more stable binding over time. Increased solvent accessibility of Mel4 also suggested its greater interaction potential with viral surfaces. Together, these findings provide a detailed mechanistic framework supporting the superior antiviral activity of Mel4 and highlight specific capsid regions that might serve as targets for peptide-based antiviral development. This study has enhanced the understanding of peptide–virus interactions at the molecular level and offers valuable insights for the rational design of next-generation antivirals against environmentally resilient, non-enveloped viruses such as adenovirus and norovirus.

B32. Antibiotic-Resistant Bacteria Isolated From Fresh Organic Vegetables Sourced From Local Producers In Eastern Spain

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Antibiotic resistance (AMR) is a critical issue within the "One Health" approach, which encompasses human and animal health, agriculture, and environmental waste management. Fresh vegetables, which are frequently consumed raw, may contribute to the spread and transmission of AMR. Therefore, the aim of this research was to study the microbiological quality and prevalence of ARB and ARG in fresh vegetables.

Twenty-one samples were purchased directly from organic farms in Valencia (Spain), and two soil samples were taken from the cultivation sites. Samples were examined for total viable bacteria, coliforms, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* spp. Isolation was performed onto culture media supplemented with cefotaxime and meropenem.

A total of 122 strains were isolated. A total of 24 Gram-negative isolates were selected and identified by biochemical tests and sequencing of 16S rRNA. Their sensitivity to 15 antibiotics was determined, and PCR assays were performed for main expanded spectrum beta-lactamase (ESBL), carbapenemase- and plasmid-mediated quinolone resistance genes, both for the samples and for the isolates.

Overall, 96% of the samples showed total viable bacteria levels over the accepted 10(5) cfu/g standard limit, and 87% showed coliforms levels over the accepted 10(4) cfu/g standard limit. In two samples (9%), *E. coli* levels were not acceptable. *Salmonella* and *L. monocytogenes* were not detected.

Seventy-nine percent of the isolates were resistant to at least one antibiotic, and 50% were ESBL productors. PCR showed the presence of bla_{CMY-2} (13%), bla_{TEM} (8%), bla_{SHV} (4%), bla_{OXA-48} (17%), bla_{KPC} (8%), bla_{IMP} (4%), *qnr*A (17%), *qnr*B (21%) and *qnr*S (8%) genes.

The detection of carbapenems resistance genes is particularly concerning because it poses a serious risk for immunocompromised patients. Thus, fresh organic vegetables harboring ARB and ARG constitute a potential risk to consumers. Further studies must be done to detect ARG and how they propagate in non-medical environments.

B33. Potential Use of Honey as an Antibiotic for *Staphylococcus Aureus*: An AFM Study

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Staphylococcus aureus causes difficult-to-treat infections with current antibiotics, often linked to resistant biofilms. Honey has the potential to act as an antibiotic due to its acidity and high sugar content. Mechanisms by which honey acts as an antibiotic are unclear. We hypothesize that exposing *S. aureus* cells to Manuka honey induces physiochemical changes to cells in a concentration-dependent manner. To test our hypotheses, Atomic Force Microscopy (AFM) was used to image cells and quantify their adhesion to a model surface. The Hertz model of contact mechanics was used to estimate the Young's moduli of the cells. *S. aureus* was exposed to 10%, 20%, 30% (MIC), and 40% honey for 2 hours. Untreated cells were the negative control. AFM was performed at room temperature on 3 cells per slide, 3 slides per culture, and 3 cultures per condition. Exposure of cells to 10%, 20% and 30% honey for 5 hours resulted in 0%, 45%, and 100% killing of cells, respectively. When cells were untreated, adhesion forces, energies, and elasticities were 17.79±39.72 pN, 4.87±5.06 aJ, and 221.19±100.22 kPa, respectively. Exposure to 10% honey enhanced adhesion forces and energies to 51.41±38.91 pN and 11.86±9.88 aJ, respectively, and did not affect elasticity. Exposure to 20% honey increased adhesion forces and energies further to 102.09±101.04 pN and 14.00±12.91 aJ, respectively, and did not affect elasticity. At 40% honey (above MIC), the adhesion forces and energies reached their maximum values at 356.22±276.98 pN and 81.07±66.37 aJ, respectively, while the elasticity dropped by 70% to 64.32±42.91 kPa. Cells were intact under all conditions investigated. The drop of elasticity could be the result of detachment of surface molecules, while increased adhesion may be associated with increased flexibility of surface molecules. Our results suggest the excellent potential for honey to be used as an antibacterial agent.

B34. 3D Printing and Functionalization to Create a Hemoperfusion Cartridge

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Every year, sepsis causes more than 11 million deaths. It can occur unpredictably during any infection, most often bacterial. It results from a dysregulation of the immune response to infection, usually causing excessive inflammation that disrupts organ function. One solution for treating this infection is hemoperfusion.

The aim of this study is to create blood filtration cartridges for hemoperfusion, grafted with polymers capable of removing bacteria and toxins from the blood in cases of bacteremia or even sepsis. We have chosen to take an innovative approach combining 3D printing and the synthesis of methacrylate-type polymers. To study the structure–activity relationship of these polymers, we synthesized copolymers of varying molar masses and compositions with amphiphilic properties, containing a hydrophobic monomer and a cationic monomer. We determined the antibacterial properties of these free copolymers in solution on Gram-negative (E. coli) and Gram-positive (S. aureus) bacteria, as well as their hemolytic activities. Tests on their anti-inflammatory properties were also conducted. The next step in the project is to functionalize a 3D-printed support with the copolymer exhibiting the best properties.

B35. Oregano–Rosemary Essential Oil Combinations as Potential Growth-Promoting Alternatives in Poultry: Evidence from Bacterial Time–Kill Curves

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The widespread use of antibiotics as growth promoters in poultry production has contributed to the emergence of antimicrobial resistance and the dissemination of zoonotic pathogens, underscoring the urgent need for effective non-antibiotic alternatives. This study aimed to evaluate the in vitro antibacterial activity of oregano (*Origanum vulgare*) essential oil (OEO), rosemary (*Salvia Rosmarinus*) essential oil (REO), and their combination (OR) against *Escherichia coli* and *Salmonella typhimurium* strains of poultry origin.

Minimum inhibitory and bactericidal concentrations were determined, and the optimal OR combination was selected using checkerboard assays. Time–kill curves were performed using ATCC reference strains (*E. coli* ATCC 25922 and *S. Typhimurium* ATCC 14028), as well as poultry field isolates (n = 2 per species), exposed to individual essential oils and their combination at different multiples of the MIC, under pH conditions simulating the avian gastrointestinal tract (pH 5.0 and 7.4). Bacterial viability was monitored over time based on viable cell counts (CFU/mL), and antibacterial activity was quantified using the antibacterial effect index (E). Results were interpreted according to established cut-off values for bacteriostatic activity ($E = 0$), bactericidal activity ($E \leq -3$), and virtual eradication ($E \leq -4$).

Both essential oils exhibited rapid and concentration-dependent antibacterial activity. At 2× and 4× MIC, OEO and REO induced immediate bacterial killing, achieving virtual eradication ($E \leq -4$) within 10–20 minutes of exposure. The OR combination consistently enhanced antibacterial activity, reaching bactericidal and eradication thresholds faster and at lower concentrations compared to individual oils. Comparable killing dynamics were observed across pH conditions and between reference strains and poultry field isolates for both *E. coli* and *S. typhimurium*.

These findings demonstrate the potent and rapid antimicrobial action of OEO and REO, particularly when used in combination, supporting their potential application as non-antibiotic growth-promoting alternatives in poultry production within a One Health framework.

B36. Underrepresented Sequence–Derived Peptides as a Novel Antimicrobial Platform to Mitigate Resistance Development

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Antimicrobial resistance poses a growing global challenge, necessitating the development of novel antibacterial strategies. One promising approach is based on short amino acid motifs that are significantly underrepresented or absent in bacterial proteomes (URS).

Using a computational strategy developed in our laboratory, Navon et al. (PNAS 2016) identified URSs by detecting patterns missing from proteomic datasets. These sequences are often species-specific and, in some cases, completely absent from bacterial proteomes. Introduction of a URSs can lead to translational arrest and even cell death. These sequences are not URSs in the human proteome, suggesting potential use as antibiotics.

Two URSs shown to induce translational inhibition in *E. coli* are CMY and CMYW. Based on these findings, we proposed two implementation strategies: delivery of synthetic URS-containing peptides or expression of URS-encoding sequences.

Chemically synthesized peptides, including CMY- and CMYW-based sequences, were applied to *Escherichia coli* and *Salmonella* cultures. These peptides exhibited limited antibacterial activity, likely due to restricted membrane permeability associated with their hydrophobic nature. To overcome this limitation, the peptides were conjugated to cell-penetrating peptides (CPPs), resulting in a substantial improvement in antibacterial activity. Optimized peptides, including RLLRRCMYW, exhibited MIC values in the range of 8–16 μM, comparable to those of conventional antibiotics, due to improved cellular uptake and stability. Notably, CMYW-based peptides showed enhanced activity relative to CMY, consistent with stronger ribosomal stalling and their absence from bacterial proteomes.

In addition, heterologous expression of URS-encoding sequences inhibited bacterial growth, supporting that URS motifs impair growth through disruption of translation.

Structural determination of ribosome–URSpep complexes (Tarabeh et al., in preparation) provides insight into their molecular interactions with the ribosome.

Our current work focuses on further optimizing peptide permeability and stability to enhance intracellular targeting of the ribosome and maximize antibacterial activity.

B37. Isolation and Characterization of Bacteriophages Active Against *Pseudomonas aeruginosa* Strains Isolated from Ocular Infection

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Background: Microbial keratitis (infection of the cornea, the clear membrane overlying the pupil and coloured iris in the eye) is often caused by *Pseudomonas aeruginosa*, especially in contact lens wearers. Commonly used antibiotics are failing against this bacterium as it is becoming increasingly resistant to antimicrobials. This study was conducted to isolate and characterize bacteriophages (phages) that could be used to design a phage cocktail that is active against multi-drug resistant (MDR) ocular *P. aeruginosa* isolates.

Methods: Raw untreated sewage water was used for isolation and purification of phages following the Phage-on-Tap protocol. Phage confirmation, enumeration and identification were performed by spot assay, double layer plaque assay and transmission electron microscopy (TEM), respectively. Lytic activity of phages was determined by host range evaluation against thirteen *P. aeruginosa* isolates that had previously been characterised as MDR and resistant to another older set of phages. Further characterization of new phages used one step growth curves and thermal stability.

Results: Four phages were isolated and purified. These phages produced high titres of phage, approximately 10^{10} plaqe forming untis (pfu)/ml. TEM analysis revealed that the phages belong to two families, Podoviridae and Siphoviridae. All phages were stable at temperatures from 4°C to 50°C. Host range via spot assays showed that Sullo-1, Sullo-2, Sullo-3 and Sullo-4 had lytic activity against 77%, 69%, 55% and 55% of *P. aeruginosa* strains. The latent period was around ~ 20 minutes for Sullo-1, Sullo-2 and Sullo-3 and 40 minutes for Sullo-4. The burst size of Sullo-1, Sullo-2, Sullo-3 and Sullo-4 was 38, 140, 246 and 73 pfu/infected cell, respectively.

Conclusions: The newly isolated phages had efficient lytic activity against ocular *P. aeruginosa* isolates and could be distinguished in terms of morphology, host range, thermal stability, burst size and latent period.

B38. Genome-wide CRISPRi-seq identifies essential genes modulating antibiotic resistance in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa (*Pae*) is a major opportunistic human pathogen, classified by the WHO in 2024 as a high-priority antibiotic resistant threat. It causes severe infections in immunocompromised individuals and patients with cystic fibrosis, largely due to a combination of intrinsic and acquired resistance mechanisms. CRISPR interference (CRISPRi) enables programmable, transcriptional repression of target genes and is well-suited for genome-wide interrogation of gene-drug interactions, allowing identification of essential pathways that become particularly vulnerable under antibiotic pressure. In CRISPRi, a single guide RNA (sgRNA) directs a catalytically inactive Cas9 protein (dCas9) to a target sequence adjacent to a PAM site, blocking RNA polymerase binding or elongation.

Here, we performed a genome-wide CRISPRi screen in the *P. aeruginosa* PAO1 reference strain, using an sgRNA pooled library exposed to sub-inhibitory concentrations of 12 antibiotics commonly used against Pae: aminoglycosides, β-lactams (including carbapenems), fluoroquinolones, and polymyxins. The analysis identified essential gene-drug interactions and pinpointed pathways (e.g., cell wall synthesis, cell division, fatty acids and lipopolysaccharide biosynthesis) whose repression altered antibiotic susceptibility. These genes represent potential therapeutic targets or adjuvant candidates to enhance existing antimicrobial efficacy while reducing toxicity associated with high drug doses. Overall, this platform provides a robust approach for investigating essential genes and their role in resistance, offering a promising strategy to identify pathogen vulnerabilities.

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B39. Natural compounds Interactions and Sequential Quercetin–Ciprofloxacin Application as Non-Bactericidal Strategies Against MRSA Biofilms

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Staphylococcus aureus (S. aureus) is a major global health concern and is classified by the World Health Organization (WHO) as a high-priority pathogen due to its multidrug-resistant (MDR) profile and virulence. Biofilm formation is a critical resistance mechanism, enhancing bacterial tolerance to antimicrobials up to 1000-fold. Consequently, the development of anti-biofilm therapies represents an urgent need, providing an alternative to conventional bactericidal antibiotics. Natural compounds, owing to their diverse modes of action and low resistance potential, have emerged as promising candidates. This study evaluates the antibacterial and antibiofilm activities of baicalin, quercetin, and 18β-glycyrrhetinic acid—individually and in combination—against methicillin-resistant *S. aureus* (MRSA) isolates. A panel of community-associated MRSA (CA-MRSA) isolates was subjected to microbiological assays, assessing effects on bacterial growth, biofilm inhibition, and biofilm eradication. Combination studies were further performed; including sequential models with thymoquinone and ciprofloxacin to assess synergistic effects. None of the tested compounds exhibited strong bactericidal activity against planktonic MRSA cells. However, baicalin and quercetin showed notable biofilm inhibition, whereas 18β- glycyrrhetinic acid demonstrated minimal effect. Quercetin exhibited a potent and dose-dependent antibiofilm activity. Combination assays revealed that quercetin–baicalin and quercetin–18β-glycyrrhetinic acid pairings produced additive inhibitory effects across MRSA strains. In contrast, the quercetin–thymoquinone combination displayed antagonistic behavior, possibly due to overlapping mechanisms of action. None of the tested compounds significantly eradicated pre-formed biofilms. The sequential treatment of an inhibitor followed by an eradicating agent has shown significant biofilm reduction in comparison to that of each compound solely. These findings demonstrate that natural compounds, particularly quercetin-based combinations, can effectively inhibit MRSA biofilm formation through non-bactericidal mechanisms. Such approaches may reduce selective pressure for resistance and offer complementary strategies to existing antibiotics. Future research should focus on elucidating molecular mechanisms of action and improving compound bioavailability and delivery to advance the translational potential of natural anti-biofilm therapies.

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