

EURESTOP GENERAL MEETING

(Grant Period 3)

30-31 January 2025, Seville (Spain)

Organized in collaboration with the Pablo De Olavide University, Seville.



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CA21145 meeting venue



The CA21145 meeting will take place in the building 31 “Parainfo” of Pablo de Olavide University Campus, which is located at Carretera de Utrera s/n, 41013, Seville, Spain

Transport from airport to University Campus

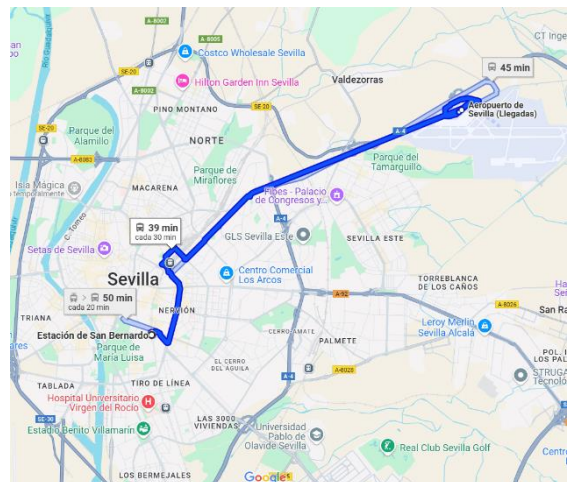
TAXI

Trip: 15-20 min; price: 30 to 35 euros (single trip)

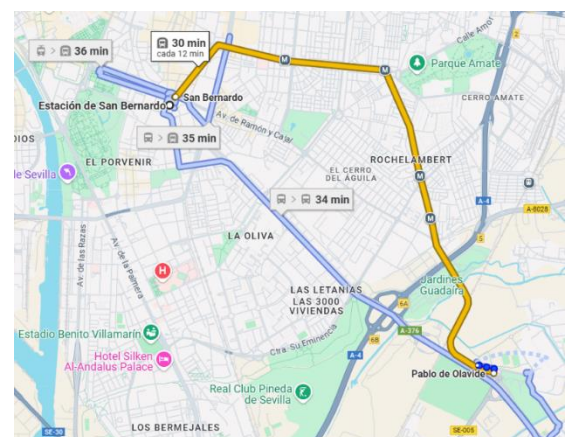
Bus and Metro

You can reach Pablo Olavide University from San Bernardo Station, which serves as a hub for buses, trains, and the metro.

Bus from Seville airport to San Bernardo Station (around 40 min; price: 5 euros for single trip).



Metro from San Bernardo Station to Pablo de Olavide University (around 40 min; price: 1.8 euros for single trip).



Thursday 30 January 2025 **Building 31 Parainfo**

LINK for remote connection: <https://eu.bbcollab.com/guest/02b9864fdfb3447cbe07ead07ed6b989>

8:15-9:00 **Registration of participants**

9:00-9:30 Welcome by Pablo de Olavide University Rector (**Francisco Oliva Blázquez**), Vice-Rector for Research, Transfer and Doctorate (**Antonia Jiménez**), Andalusian Centre of Developmental Biology Director (**Peter Askjaer**), and EURESTOP Chair (**Mattia Mori**).

Session #1. Chairs: Younes Smani & Vera Isca

9:30-10:10 **Keynote Lecture**

Jesús Rodríguez Baño, Virgen Macarena Hospital, Seville University, Spain: *Clinical research in antimicrobial resistance: medical needs, challenges and novel approaches.*

10:10-10:25 **Benedetta Pellegrini**, University "G. d'Annunzio" of Chieti-Pescara, Italy: *Healthcare Surveillance of Antimicrobial Resistance in Bacterial Species Belonging to Enterobacteriaceae.*

10:25-10:40 **Marko Jelovac**, University of Belgrade, Serbia: *Leveraging architecture and design to combat antimicrobial resistance: how to enhance space and visual strategies.*

10:40-10:55 **Tomislav Meštrović**, University North, University centre Varaždin, Croatia: *Regional trends in the burden of antimicrobial resistance (AMR) in Europe from 1990 to 2021: insights from the Global Burden of Disease GRAM project.*

10:55-11:55 **Coffee break + poster session**

Session #2. Chairs: Carole Devaux & Bilal Javed

11:55-12:10 **Martijn Riool**, University Hospital Regensburg, Germany: *Galleria mellonella larvae: a promising animal model to study biofilm maturation in orthopaedics infections.*

12:10-12:25 **Luís D. R. Melo**, University of Minho, Portugal: *The Multifaceted Relationship Between Phages and Biofilms: Lessons from S. epidermidis.*

12:25-12:40 **Marta Pulido Sánchez**, Universidad Pablo de Olavide, Spain: *Decision-making in the Pseudomonas lifestyle: HsbA oppositely regulates biofilm formation in P. putida and P. aeruginosa.*

12:40-12:55 **Raivis Žalubovskis**, Latvian Institute of Organic Synthesis, Latvia: *Case Study on the Antimicrobial and Antibiofilm Properties of Latvian Propolis Extracts in Various Solvents.*

12:55-14:30 **Lunch break "Cafetería Plaza de América"**

Session #3. Chairs: Cristina Nativi Daniel Ziental

- 14:30-14:45 **Silvia Cammarone**, Sapienza University of Rome, Italy: *Exploring the Structure-Activity Relationships of Dehydroabietic Acid Derivatives as Promising Scaffolds for Developing ArnT-Mediated Colistin Resistance Inhibitors.*
- 14:45-15:00 **Agnese Brangule**, Riga Stradiņš University, Latvia: *Case Study on Enhancing Efficiency and Effort in High Hydrostatic Pressure Sterilization.*
- 15:00-15:15 **Irene Molina Panadero**, Universidad Pablo de Olavide, Spain: *A novel antibiotic class targeting the enolase of Acinetobacter baumannii.*
- 15:15-15:30 **Betül Giray**, Istinye University, Turkey: *Antimicrobial Properties and In Silico Studies of some N-acyl hydrazone and 2,5 substituted 1,3,4-oxadiazole derivatives.*
- 15:30-15:45 **Vera M.S. Isca**, Universidade Lusófona, Portugal: *Exploring Abietane Diterpenoids as Potential Antimicrobial Agents Against Resistant Bacteria.*
- 15:45-16:00 **Simone Carradori**, "G. d'Annunzio" University of Chieti-Pescara, Italy: *Modulation of bacterial Carbonic Anhydrases: a case study in MedChem.*

16:00-17:00 **Poster session + coffee break + GROUP PICTURE**

17:00-18:30 **MC meeting (reserved to MC members/delegates)**

Link for remote connection (MC members only):

<https://eu.bbcollab.com/guest/f551df6130e94668922e65e1a512dc81>

21:00 **Social Dinner**

Friday 31 January **Building 31 Parainfo**

Link for remote connection: <https://eu.bbcollab.com/guest/ada31255b35a412ead525d6508e3893b>

Session #4. Chairs: Rossella Grande & Martijn Riool

- 9:00-9:40 **Keynote Lecture**
Juan José Infante Viñolo, Vaxdyn (Vaccines & Antibodies against Resistant Infections): *Spanish start-ups providing solutions to the Anti-Microbial Resistance crisis, the Vaxdyn case of funding early pharmaceutical development.*
- 9:40-9:55 **Marta Vicente-Pazos**, Universitat de Girona, Spain: *Comparison of protective efficacy of two DNA vaccines against Klebsiella pneumoniae virulence factors.*
- 9:55-10:10 **Muhammad Asaduzzaman**, University of Oslo, Norway: *Spatiotemporal Distribution and Diversity of Airborne Resistant Bacteria: An Exploratory One Health Study in the Urban and Rural Environments of Bangladesh.*
- 10:10-10:25 **Pusta Orujova**, Azerbaijan Medical University, Azerbaijan: *The Role of HMGB1 Gene Variations in Diagnosing Sepsis in Newborns Admitted to the NICU.*
- 10:25-10:40 **Fintan Kelleher**, Centre for AMR and One-Health Research, Ireland: *Do Antibiotic Metabolites have a Role in the Development of Antibiotic Resistance?*

10:40-11:40 **Coffee break + poster session**

Session #5. Chairs: Priyanka Sahariah & Luis Melo

- 11:40-11:55 **Vladimír Křen**, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic. *New Nitrogen-Containing Flavonoids Suppress Gentamicin Resistance of Staphylococcus aureus.*
- 11:55-12:10 **Jovana Francuz**, University of Novi Sad, Serbia: *Lactones as Antimicrobial Agents: From Synthesis to Evaluation.*
- 12:10-12:25 **Ricardo J.F. Ferreira**, Universidade de Lisboa, Portugal: *Exploring 5-membered ring spiro p53 activator compounds as antibacterial agents.*
- 12:25-12:40 **Marialaura Marchetti**, University of Parma, Italy: *DEfeat Antimicrobial Resistance through Iron Starvation in Staphylococcus aureus (ERASE): how biochemistry can support the search of novel antimicrobials.*
- 12:40-12:55 **Ilaria D'Agostino**, University of Pisa, Italy: *Membrane-Active AlkylGuanidino Ureas Against Bacteria: An Intriguing Tale.*
- 12:55-13:10 **Calum Haydon**, University of Bristol, UK: *C2-Linked Arabinose-Functionalized Polystyrene Microbeads Selectively Target Staphylococcus aureus.*

13:10-14:30 **Lunch break "Cafetería Plaza de América"**

Session #6. Chairs: Patricia Rijo & Epole Ngolle Ntungwe

- 14:30-14:45 **Magdalena Zajac**, National Veterinary Research Institute, Poland: *Occurrence and molecular characteristics of cephalosporin-resistant Escherichia coli isolated from goose farms in Poland.*
- 14:45-15:00 **Violeta Santrač**, Veterinary Institute of the Republic of Srpska "Dr. Vaso Butozan" Banja Luka, Bosnia and Herzegovina: *Drug-resistant bacteria from honey jar, do you want to eat them?*
- 15:00-15:15 **Abidelfatah M. Nasser**, Beitberl College of Education, Israel: *Abundance of Antibiotic Resistant Genes (ARGs) in Wastewater and Receiving Surface Water.*
- 15:15-15:30 **Stephen J. Fey**, CelVivo ApS, Denmark: *Multidrug resistance in the lab and in vivo: The growing role of 3D Cell Culture in identifying commercially valuable compounds.*
- 15:30-15:45 **Daniel Ziental**, Poznan University of Medical Sciences, Poland: *Quaternized phthalocyanines as a powerful tool against a broad spectrum of pathogenic microorganisms and melanoma.*
- 15:45-16:00 **Basak Kayitmazer**, Boğaziçi University, Turkey: *Rheology and Thermodynamics of Condensates from Ionic Polysaccharides.*
- 16:00-16:15 **Viktorová Jitka**, University of Chemistry and Technology Prague, Czech Republic: *Adjuvant therapy - an effective tool to address antibiotic resistance.*
- 16:15-16:30 EURESTOP vice-chair (**Patricia Rijo**) and Meeting organizer (**Younes Smani**)
Concluding remarks and next events

ABSTRACTS (Keynote Lecturers)

Clinical research in antimicrobial resistance: medical needs, challenges and novel approaches

Jesús Rodríguez Baño

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Clinical research in treatment of infections caused by antimicrobial resistant bacteria is jeopardized by the heterogeneity of infections characteristics and patients' features, all of which may influence outcomes and therefore be confounding factors.¹ While most studies are observational in design, the problems of confounding and immortal time biases are frequently not adequately addressed. Of course, randomized trials are the best design to compare treatment options, but they also face relevant challenges. Patients with infections caused by multidrug-resistant bacteria are frequently aged, with an important burden of comorbidities and sometimes suffering from acute severe illness, making it difficult to recruit them for trials. Also, delays in obtaining the susceptibility results using conventional methods is also a problem. Finally, sometimes there is not a unique drug that can be considered as the standard comparator. During last years, some initiatives are being developed to improve the development of trials in this field, including the use of rapid diagnostic test, pragmatic designs, adaptative platform trials² and trials with personalized randomization for options suitable for each specific patient.³

References

1. Bettiol E, Rottier WC, Del Toro MD, Harbarth S, Bonten MJ, Rodríguez-Baño J; COMBACTE consortium. Improved treatment of multidrug-resistant bacterial infections: utility of clinical studies. *Future Microbiol.* 2014;9(6):757-71. doi: 10.2217/fmb.14.35.
2. Lanini S, Ioannidis JPA, Vairo F, et al. Non-inferiority versus superiority trial design for new antibiotics in an era of high antimicrobial resistance: the case for post-marketing, adaptive randomised controlled trials. *Lancet Infect Dis.* 2019 Dec;19(12):e444-e451. doi: 10.1016/S1473-3099(19)30284-1.
3. Walker AS, White IR, Turner RM, et al. Personalised randomised controlled trial designs-a new paradigm to define optimal treatments for carbapenem-resistant infections. *Lancet Infect Dis.* 2021 Jun;21(6):e175-e181. doi: 10.1016/S1473-3099(20)30791-X.

Spanish start-ups providing solutions to the Anti-Microbial Resistance crisis, the Vaxdyn case of funding early pharmaceutical development

Juan José Infante Viñolo^a

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In 2016, the O'Neill report on antimicrobial resistance (AMR) outlined potential solutions to address the AMR crisis, all of which were at a very early stage. The report warned that if these solutions were not implemented promptly, AMR could cause more than 10 million deaths per year globally by 2050, surpassing cancer as a leading cause of mortality. Since then, there has been both bad news and good news. The bad news came with the GRAM report in 2022 and subsequent updates, which revealed that the impact of AMR is even greater than anticipated, with nearly 8 million deaths already occurring annually. However, the good news is that many of the early-stage solutions mentioned in the O'Neill report have now progressed to the clinical stage and are much more advanced. While significant investment is still required to bring these solutions to market, the drugs have been optimized, and the key barriers to implementation have been identified. Additionally, it has become evident that these solutions will largely be delivered by small biotechnological start-ups. Vaxdyn is one such example. In this talk, we will explore the funding sources and challenges involved in bringing to market one of the critical solutions to the AMR crisis: vaccines for the prevention of diseases caused by AMR bacteria in at-risk populations.

References

1. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. London: Review on Antimicrobial Resistance, 2016.
2. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 2022; 399: 629–55.
3. GBD 2021 Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. *Lancet* 2024; 404: 1199-1226.
4. Rodríguez-Rosado et al. K-Vax, a new vaccine for prevention of disease by *Klebsiella pneumoniae* with potential for global strain coverage. KLEBSIELLA EPIDEMIOLOGY AND BIOLOGY SYMPOSIUM 2024 November 20th-22nd, 2024 Institut Pasteur, Paris, France

ABSTRACTS (in order of presentation)

Healthcare Surveillance of Antimicrobial Resistance in Bacterial Species Belonging to Enterobacteriaceae

Benedetta Pellegrini^a, Valentina Puca^a, Beatrice Marinacci^a, Giorgia Stornelli^{a,b}, Martina D'Alfonso^a, Pamela Di Giovanni^c, Roberta Z. Marulli^d, Ivana Cataldo^d, Rossella Grande^a.

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Enterobacteriaceae are a family of Gram-negative bacteria, widely distributed in both the environment and the gastrointestinal tract of humans and animals. However, several members are recognised as opportunistic pathogens and can cause a range of nosocomial infections, including bacteraemia, urinary tract infections, and pneumonia [1]. Genera of clinical relevance include *Escherichia*, *Klebsiella*, and *Proteus*, especially due to their ability to develop antimicrobial resistance, often mediated by the production of Extended-Spectrum Beta-Lactamases (ESBLs) and carbapenemases (CREs) [2 – 3]. Antimicrobial resistance represents one of the major threats to global public health. The aim of this retrospective study is to analyse the antimicrobial resistance profiles of bacteria species belonging to the Enterobacteriaceae family, with particular focus on clinically relevant species such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*.

The investigation has examined biological samples collected between 2018 and 2023 at the Microbiology Laboratory of San Pio Hospital in Vasto (Chieti, Italy). The analysis included a total of 890 patients, of whom 41.6% were hospitalised and 58.6% non-hospitalised, with a mean age of 73 years. The microorganisms isolated from the biological samples, predominantly urine cultures (79.2%), were obtained using culture-based methods, identified via biochemical tests, and analysed for their antimicrobial susceptibility patterns using the Walk Away automated system. The most frequently detected bacterial species were *Escherichia coli* (63.17%), *Klebsiella pneumoniae* (21.02%), and *Proteus mirabilis* (8.15%). All isolated species exhibited at least one resistance to the tested antimicrobials, with the majority showing resistance to more than four antimicrobials. Specifically, 21.7% of the species were resistant to five antimicrobials, 18.1% to six, and 15.7% to seven antimicrobials. The antimicrobial class with the highest resistance rates was third-generation cephalosporins (cefotaxime, 15.96%), followed by ampicillin (15.57%) and ciprofloxacin (13.18%).

The growing antimicrobial resistance developed by these microorganisms pose a significant clinical challenge, highlighting the need for advanced diagnostic tools and therapeutic options. The results of this study aim to provide useful data to support therapeutic decisions and improve the prevention of bacterial infections using new control strategies.

References

1. Janda, J. M., & Abbott, S. L. (2021). The Changing Face of the Family Enterobacteriaceae (Order: "Enterobacterales"): New Members, Taxonomic Issues, Geographic Expansion, and New Diseases and Disease Syndromes. *Clinical Microbiology Reviews*, 34(2), e00174-20.
2. Lahlaoui, H., Ben Haj Khalifa, A., & Ben Moussa, M. (2014). Epidemiology of Enterobacteriaceae producing CTX-M type extended spectrum β -lactamase (ESBL). *Medecine et Maladies Infectieuses*, 44(9), 400–404.
3. Ma, J., Song, X., Li, M., Yu, Z., Cheng, W., Yu, Z., Zhang, W., Zhang, Y., Shen, A., Sun, H., & Li, L. (2023). Global spread of carbapenem-resistant Enterobacteriaceae: Epidemiological features, resistance mechanisms, detection and therapy. *Microbiological Research*, 266, 127249.

Leveraging architecture and design to combat antimicrobial resistance: how to enhance space and visual strategies

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Antimicrobial resistance (AMR) represents a pressing global health threat, exacerbated by inappropriate use of antimicrobial agents and inadequate attention to environmental and social determinants of health. Architectural strategies that enhance infection prevention and control (IPC) include designing energy-efficient housing with advanced ventilation systems, integrating antimicrobial materials for high-touch surfaces, as well as employing adaptable, sustainable urban planning methods. Likewise, urban housing innovations – such as plus-energy residential models – provide examples of how energy-efficient designs can improve indoor environments, reduce respiratory infections and minimize health inequities in vulnerable populations. By reimagining our space as an active player in frontline defense, we can exert downstream effects in reducing the overall burden of AMR.

Healthcare facilities also serve as critical environments where architectural interventions can mitigate the spread of resistant pathogens. Incorporating natural ventilation, optimizing daylight access and designing flexible spaces tailored to healthcare demands are essential components of sustainable hospital architecture. These interventions align with the 'One Health' framework, which integrates human, animal and environmental health to address AMR comprehensively. Complementing architectural innovations, the design of visual identities (such as the EURESTOP logo) often serves as a seed in raising awareness and galvanizing action against AMR. By applying design principles that prioritize clarity and engagement, visual strategies not only increase visibility, but also strengthen public advocacy and prompt stakeholder collaboration. The research clearly shows the need for cross-sectoral partnerships to integrate architectural advancements with visual communication efforts. Through an interdisciplinary approach, both sustainable urban planning and design can reshape environments to promote health equity and reduce the impact of infectious diseases – but its propensity to address the growing challenge of AMR is increasingly being recognized.

Regional trends in the burden of antimicrobial resistance (AMR) in Europe from 1990 to 2021: insights from the Global Burden of Disease GRAM project

Tomislav Meštrović^{a,b}, Gisela Robles Aguilar^c, Authia Gray^a, Lucien Swetchinski^a, Kevin Shunji Ikuta^a, Erin Chung^a, Chieh Han^a, Eve Wool^a, Anna Gershberg-Hayoon^a, Stein Emil Vollset^a, Daniel T Araki^a, Nicole Davis Weaver^a, Ben Cooper^c (Project Leadership, University of Oxford), Christopher J.L. Murray^a (Project Leadership, IHME/UW), Mohsen Naghavi^a (Project Leadership, IHME/UW)

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Antimicrobial resistance (AMR) represents a growing public health threat globally, with significant regional variation. This study examines the burden of AMR in different European regions from 1990 to 2021, leveraging data from the most recent Global Burden of Disease (GBD) GRAM project, which included 22 bacterial pathogens and 84 pathogen-drug combinations. Using counterfactual scenarios – one replacing drug-resistant infections with susceptible ones and another assuming no infection – AMR burden was quantified in terms of deaths and disability-adjusted life-years (DALYs).

In 2021, Western Europe exhibited the highest burden, with 212,800 (95% UI 185,500–240,100) deaths associated with AMR and 46,100 (40,000–52,100) deaths attributable to AMR. Eastern Europe had 145,000 (127,000–163,000) deaths associated with AMR and 35,600 (30,100–41,100) deaths attributable to AMR in 2021, which is an increase when compared to 1990. Central Europe reported 80,200 (71,400–89,100) deaths associated with AMR and 18,700 (16,500–20,800) attributable deaths. Methicillin-resistant *Staphylococcus aureus* (MRSA) and aminopenicillin-resistant *Escherichia coli* emerged as leading pathogen-drug combinations for attributable and associated deaths, with substantial variation in other pathogen-drug combinations among the regions. Resistance to carbapenems in Gram-negative bacteria, particularly *Klebsiella pneumoniae*, has significantly increased in all regions, with the highest growth observed in Eastern Europe. There is also a significant variation of AMR burden in accordance with age, with older individuals being impacted more.

The findings highlight alarming trends of AMR, particularly in older populations, reflecting demographic shifts and healthcare disparities. Effective interventions – including tailored antimicrobial stewardship in accordance with regional specificities, vaccine development and equitable access to healthcare resources – are urgently needed. This regional comparison underscores the need for customized policies to address the rising threat of AMR in Europe by 2050.

References

1. Naghavi M, Vollest SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, Robles Aguilar G, Meštrović T, et al. Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050. *The Lancet*. 2024;404(10459):1199-1226. doi: 10.1016/S0140-6736(24)01867-1.

***Galleria mellonella* larvae: a promising animal model to study biofilm maturation in orthopaedics infections**

Raphaëlle Youf^a, Ruth Schewior^a, Gopala Mannala^a, You Zhao^a, Volker Alt^a, **Martijn Riool^a**

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In trauma surgery, the development of biomaterial-associated infections (BAI) is one of the most common complications affecting trauma patients, requiring prolonged hospitalization and the intensive use of antibiotics. Following the attachment of bacteria on the surface of the biomaterial, the biofilm-forming bacteria could initiate a chronic implant-related infection. Despite the use of conventional local and systemic antibiotic therapies, persistent biofilms involve various resistance mechanisms that contribute to therapeutic failures. The development of *in vivo* chronic BAI models to optimize antibiofilm treatments is a major challenge. Indeed, the biofilm pathogenicity and the host response need to be finely regulated, and compatible with the animal lifestyle. Previously, a *Galleria mellonella* larvae model for the formation of an early-stage biofilm on the surface of a Kirschner (K)-wire was established.¹ In the present study, two models of mature biofilm using clinical *Staphylococcus aureus* strains were assessed: one related to contaminated K-wires (*in vitro* biofilm maturation) and the second to hematogenous infections (*in vivo* biofilm maturation). Rifampicin was used as a standard drug for antibiofilm treatment.

In the first model, biofilms were formed following an incubation period (up to 7 days) in the CDC Biofilm Reactor (CBR, BioSurface Technologies). Then, after implantation of the pre-incubated K-wire in the larvae, rifampicin (80 mg/kg) was injected and the survival of the larvae was monitored. In the second model, biofilm formation was achieved after an incubation period (up to 7 days) inside the larvae and then, after removing the K-wires from the host, *in vitro* rifampicin susceptibility assays were performed (according to EUCAST).

The first model indicates that *in vitro* biofilm maturation affects the bacterial pathogenicity in the host, depending on the *S. aureus* strain used. Furthermore, the more the biofilm is matured, the more the rifampicin treatment efficiency is compromised. The second model shows that, despite the fast *in vivo* biofilm formation in the host, the number of bacteria, either attached to the surface of the K-wire surface or in surrounding tissue of the larvae, was not increased over time.

Altogether, these results allow the establishment of biofilm models using *G. mellonella* larvae in order to understand the impact of biofilm maturation on both the bacterial pathogenicity and the efficiency of antibiofilm treatments.

1. Y. Zhao *et al.*, *Antibiotics* **2024**, 13, 692, DOI: 10.3390/antibiotics13080692

The Multifaceted Relationship Between Phages and Biofilms: Lessons from *S. epidermidis*

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In natural environments, bacteria predominantly exist outside the exponential growth phase. This poses a challenge for antimicrobial research, as the efficacy of agents, including phages, is often tested against rapidly dividing bacterial cells. Yet, most bacterial biomass exists in the form of biofilms, which are highly tolerant to antimicrobials due to the protective biofilm matrix and the low metabolic activity of resident cells. Despite their promise, relatively few staphylococcal phages have shown consistent efficacy against biofilms or the ability to infect metabolically inactive cells.

In my presentation, I will show different studies of the interactions between phages and biofilms, giving more detail in the interaction of *Staphylococcus epidermidis* with phage SEP1. SEP1 demonstrated limited activity against intact biofilms, with the biofilm matrix identified as the primary barrier to phage efficacy. However, SEP1 displayed remarkable effectiveness against biofilm-dispersed cells, persister cells, and stationary-phase cells¹. Through RNA-seq analysis, SEP1 was shown to hijack the transcriptional machinery of its host, reactivating key metabolic and biosynthetic pathways in stationary-phase cells to enable successful replication².

These findings underscore the complexity of phage-biofilm interactions and highlight the therapeutic potential of phages capable of targeting dormant cells. This study provides valuable insights for advancing phage therapy, particularly for addressing biofilm-related chronic infections where traditional antimicrobials often fall short.

KEYWORDS: bacteriophages; biofilms; stationary-phase cells; phage/bacteria interactions; RNA seq

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References

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2. Silva MD, Pinto G, França A, Azeredo J, Melo LDR. 2024. Phage SEP1 hijacks *Staphylococcus epidermidis* stationary cells' metabolism to replicate. *mSystems* 9:e00263-24. <https://doi.org/10.1128/msystems.00263-24>

Decision-making in the *Pseudomonas* lifestyle: HsbA oppositely regulates biofilm formation in *P. putida* and *P. aeruginosa*

Marta Pulido Sánchez^a, Elisa Montero-Beltrán^a, Aroa López-Sánchez^a, Fernando Govantes^a.

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Life cycles of most bacteria are characterized by the alternation of a single cell-based planktonic stage and a sessile stage during which they develop highly structured surface-associated communities known as biofilms. The biofilm lifestyle is often induced as a form of stress response that confers resistance from host defences and antimicrobial treatments. While this is true for the opportunistic pathogen *Pseudomonas aeruginosa*, biofilm formation in the soil bacterium *Pseudomonas putida* occurs, by contrast, under nutrient-sufficient conditions, with biofilm dispersal triggered by nutritional stress.

HptB, HsbR and HsbA are components of a signal transduction pathway that is widely conserved in the genus *Pseudomonas*. HsbA is an anti-sigma factor antagonist that promotes biofilm formation in *P. aeruginosa* by stimulating the diguanylate cyclase (DGC) activity of HsbD [1]. In sharp contrast to the output of this regulatory network described in this species, our work unveils that the HptB-HsbR-HsbA pathway represses biofilm formation in *P. putida* [2].

Deletion of *hsbA* provoked the resumption of biofilm formation after dispersal in late stationary phase, stimulated medium-air pellicle formation, and enhanced Congo Red adsorption. These phenotypes were traced down to increased c-di-GMP levels during stationary phase dependent on the DGC activity of CfcR and its cognate sensor kinase, CfcA. The CfcA-CfcR two-component system is conserved in environmental *Pseudomonas*, but absent in *P. aeruginosa* [3].

The activity of HsbA in *P. aeruginosa* is regulated by phosphorylation at the serine-56 residue by the HptB-HsbR phosphorelay pathway. In *P. putida*, expression of non-phosphorylatable and phosphomimetic mutant HsbA proteins showed that HsbA phosphorylation conditioned its interaction with CfcR and CfcA, and the intracellular distribution of the three proteins. Phenotypes of the Δ *hsbR* and Δ *hptB* mutants supported a role of HsbR and HptB in the phosphorylation of HsbA.

Our findings support a model in which HsbA forms a complex with CfcR to inhibit its DGC activity. This system contributes to biofilm dispersal by denying biofilm formation, but allows quick onset of de novo biofilm formation in response to physiological cues that release HsbA inhibition to restore CfcR DGC activity. This work highlights how slight variations in the regulatory networks of closely-related species can drive drastically different responses to fit their particular lifestyles.

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Case Study on the Antimicrobial and Antibiofilm Properties of Latvian Propolis Extracts in Various Solvents

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Propolis, a resinous substance collected by bees from plant exudates, has gained recognition for its potent antimicrobial properties. This study investigates the antimicrobial efficacy of propolis sourced from three locations in Latvia, focusing on its activity against Gram-positive and Gram-negative bacteria, clinical isolates, and fungi. Propolis was extracted using six different solvents — acetonitrile (MeCN), ethanol (EtOH), methyl tert-butyl ether (MTBE), dichloromethane (DCM), ethyl acetate (EtOAc), and hexane (HeX) — to explore solvent-specific activity profiles. The extracts and raw propolis were tested against *Escherichia coli* (EC) ATCC 25922, *extended-spectrum beta-lactamase-producing E. coli* (ESBL), *Staphylococcus aureus* (SA) ATCC 25923, *methicillin-resistant Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* (PA) ATCC 27853 and *Candida albicans* (CA) ATCC 10231.

Antimicrobial activity was assessed through well-diffusion assays, measuring inhibition zones, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests were conducted to evaluate the potency of the extracts. Additionally, biofilm inhibitory properties were examined using biofilm assays. Results demonstrated that all extracts exhibited notable antimicrobial effects, particularly against Gram-positive bacteria (SA and MRSA), Gram-negative bacteria (PA) and CA fungi. All extracts showcased the most significant activity against CA. Polar solvent extracts (EtOH, MeCN and EtOAc) showed the most significant activity in the well-diffusion and MIC assays, suggesting superior extraction of bioactive compounds. Meanwhile, MTBE and DCM extracts also displayed strong antimicrobial effects.

Biofilm formation poses a significant challenge in chronic and drug-resistant infections. All extracts disrupted biofilm formation in SA, MRSA, and CA, indicating propolis's potential as a biofilm-inhibitory agent. This finding highlights the potential of propolis as a source of biofilm-inhibitory agents.

This study highlights the broad-spectrum antimicrobial activity of propolis, particularly against multidrug-resistant pathogens like MRSA and CA. The varying effectiveness of extracts points to the importance of solvent selection in recovering bioactive compounds, offering insights for developing propolis-based antimicrobial therapies. Further research is needed to identify the specific bioactive compounds and their mechanisms of action. Propolis extracts show promise as natural, sustainable alternatives to synthetic antibiotics in combating biofilm-associated infections and multidrug-resistant pathogens.

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Exploring the Structure-Activity Relationships of Dehydroabietic Acid Derivatives as Promising Scaffolds for Developing ArnT-Mediated Colistin Resistance Inhibitors

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Colistin is a last-resort antibiotic for treating multidrug resistant Gram-negative bacterial infections. However, in recent years, the occurrence of resistance phenomena related to using such antibiotics has been documented.¹ In *Pseudomonas Aeruginosa*, the molecular mechanism that gives resistance to colistin has to be mainly ascribed to a glycosyltransferase enzyme, named ArnT.² While our earlier work identified the ent-beyerene diterpene FDO as a promising ArnT inhibitor,³ its limited natural abundance, complex purification, and lack of chromophore groups posed challenges for further development. Recently, to overcome these limitations, we simplified the complex structure of ent-beyerene to optimize the hit compound FDO by minimizing the crucial pharmacophore structures necessary for biological activity. We investigated structure-activity relationships (SARs) around the aromatic abietane scaffold of podocarpic acid (PD1), which lacks the ring D compared to the ent-beyerene one, maintains the same configuration at C-4 with respect to FDO, and features an aromatic ring C and a hydroxyl group at the C-12 position. Semisynthetic derivatives of PD1 were synthesized, and microbiological studies identified effective colistin adjuvants while revealing key functional groups required for ArnT inhibition. Based on these findings, to validate the abietic scaffold as a promising platform for developing ArnT inhibitors, we extended our SAR studies to dehydroabietic acid (DH-1), a structurally related diterpene with distinct features, including an isopropyl group at C-13, an opposite stereochemistry at C-4 compared to PD1, and the absence of a phenolic group at C-12. *In silico* studies confirmed that the aromatic portion of DH-1 interacts favorably with the aromatic residues of the ArnT binding site, enhancing its complementarity with the target. Guided by these new insights, a focused library of 21 DH-1 derivatives was synthesized, incorporating functional groups previously identified as essential for ArnT inhibition. The modifications explored included (i) the oxalyl group at C-18, (ii) variations in alkyl chain length and flexibility, (iii) stereochemical orientation at C-4, (iv) electron-donating or withdrawing groups at C-12, (v) substitution of isoprenyl with acetyl at C-13, (vi) replacement of the aromatic system with a conjugated diene, and (vii) substitution of oxygen with nitrogen at C-18. This study highlights the versatility and potential of the abietane scaffold, with both PD1 and DH-1 serving as cost-effective platforms for the development of potent ArnT inhibitors to counteract colistin resistance.

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Case Study on Enhancing Efficiency and Effort in High Hydrostatic Pressure Sterilization

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Achieving sterility is vital for biomaterials intended for implantation or contact with living tissues, particularly in applications such as tissue replacement, bone defect repair, and wound healing. This study explores High-Pressure Processing (HHP) as an innovative sterilization method for hydrogel-based biomaterials, addressing the critical need for practical sterilization approaches that maintain material integrity and minimize resource use. HHP has emerged as a superior alternative to conventional sterilization techniques—including thermal, radiation, chemical, and sterile filtration—by efficiently deactivating microorganisms at lower energy levels (250 kJ/kg) while preserving the properties of sensitive substrates.

Our research aimed to optimize HHP sterilization parameters—pressure, treatment duration, cycle oscillation, and volume of bacterial suspension—while introducing the output parameter “effort.” This metric evaluates sterilization efficiency by correlating the desired microbial deactivation with minimized input parameters, effectively enhancing resource utilization. Using *Escherichia coli* as a model organism, we examined the susceptibility of this barosensitive Gram-negative bacterium to HHP, whose outer membrane allows for easier disruption under high pressure compared to Gram-positive counterparts.

The experimental framework consisted of parameter parametrization, wherein we established input variables (pressure range: 100-300 MPa; holding times: 5-15 minutes; cycle counts: 1-5) and output measures (colony-forming units – CFU). The studies were executed with a state-of-the-art pressure vessel at ambient temperature. To refine our methodology, we implemented Genetic Algorithms (GAs) for data generation and Monte Carlo Simulations (MCS) to propose 500 novel experimental scenarios, significantly streamlining the experimental process through machine learning integration. Our findings indicate that the proposed framework optimizes multi-parameter sterilization processes, ensuring reliable outcomes and reduced resource demands. However, further exploration is necessary to validate this method’s efficacy across a range of Gram-positive and Gram-negative bacteria, and various biocompatible materials, particularly hydrogels. Such advancements could broaden the applicability of HHP sterilization in diverse biomedical fields.

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A novel antibiotic class targeting the enolase of *Acinetobacter baumannii*

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The emergence of MDR *Acinetobacter baumannii* represents a critical global health threat, particularly when resistance arises against colistin, often regarded as a last-resort antibiotic. This study aimed to investigate the antibacterial properties of ENOblock, a compound identified through high-throughput screening of the EU-OPENSREEN library, as a potential treatment for MDR *A. baumannii*.

In vitro assays were conducted to evaluate ENOblock's antibacterial activity using microdilution assays, time-kill curves, and SYTOX Green membrane permeability tests. Bacterial cytological profiling (BCP) was employed to assess morphological changes after ENOblock treatment, shedding light on its mechanism of action. Computational docking was performed to identify the ENOblock molecular target and analyze its binding affinity to *A. baumannii* enolase. Target validation was achieved using an enolase-deficient mutant (Δeno) by measuring MIC and bacterial growth changes. Synergistic effects of ENOblock combined with antibiotics were assessed in reference and colistin-resistant strains. Finally, ENOblock's efficacy was evaluated *in vitro* in human epithelial and macrophage cells and *in vivo* in a *Galleria mellonella* model.

ENOblock demonstrated potent antibacterial activity with an MIC₅₀ of 16 mg/L against clinical carbapenem- and colistin-resistant *A. baumannii* strains, surpassing the MIC₅₀ values of colistin and imipenem/meropenem. SYTOX Green assay revealed that ENOblock caused rapid and extensive membrane damage within 10 minutes of exposure. BCP analysis revealed unique morphological changes not observed with clinically used antibiotics, suggesting a distinct mechanism. Computational docking indicated strong binding of ENOblock to enolase, which was confirmed by experiments with Δeno strain. The enolase-deficient mutant exhibited a four-fold increase in MIC and greater growth resistance to ENOblock, supporting enolase as its probable target. ENOblock exhibited synergistic effects with colistin, enhancing its efficacy against wild type and clinical strains, though no synergy was observed with other antibiotics. Furthermore, ENOblock significantly reduced *A. baumannii* adherence/invasion in epithelial and macrophage cells *in vitro* and improved survival rates in *G. mellonella* infected with *A. baumannii*, demonstrating its therapeutic potential.

These findings highlight ENOblock as a promising candidate for antimicrobial development, with the potential to combat the critical threat posed by MDR *A. baumannii* infections.

Antimicrobial Properties and In Silico Studies of some N-acyl hydrazone and 2,5 substituted 1,3,4-oxadiazole derivatives

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In recent years, increasing antimicrobial resistance in pathogenic microorganisms that cause infection has become a major problem. The reason for this, increase in bacteria is the excessive use of antimicrobial agents, genome plasticity, and resistance genes acquired through horizontal gene resistance mechanisms. Some gram-negative pathogenic microorganisms have multiple antibiotic resistance and the microorganisms in this group are called ESKAPE pathogens like *Enterococcus*, *Staphylococcus*, *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Enterobacter* and *E. coli* strains. This pathogens resistant to some classes of antibiotics such as macrolides, tetracyclines, beta lactams and beta lactamase inhibitor concentrations, as well as last line antibiotics such as glycopeptides, carbapenems and polymyxins. Due to this increased resistance in microorganisms, it has become essential to develop new antimicrobial agents, especially for the treatment of infections caused by these pathogens. Therefore, in this study, the antimicrobial effects of N-acyl hydrazones **1-15** and 2,5-substituted 1,3,4-oxadiazoles **16-27** against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, and clinically isolated *Shigella sonnei*, *Klebsiella pneumoniae* and *Candida albicans* were assessed. Kirby Bauer disc diffusion and MIC tests were used, showing that most of these compounds are active against tested microorganisms. Particularly, several compounds proved active against *E. coli*, whereas *S. aureus* showed higher resistance. Additionally, molecular docking and dynamics (MD) studies identified four compounds of these as potential inhibitors of bacterial DNA gyrase B (GyrB).

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Exploring Abietane Diterpenoids as Potential Antimicrobial Agents Against Resistant Bacteria

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Antimicrobial resistance is a significant health problem, with increasing treatment failures due to the failing efficacy of current antibiotics. Notably, two abietane diterpenoids isolated from different species of *Plectranthus*, 7 α -acetoxy-6 β -hydroxyroyleanone (**Roy**) and 6,7-dehydroroyleanone (**DeRoy**), have demonstrated promising antibacterial activity, particularly against methicillin-resistant *Staphylococcus aureus* (MRSA) strains.^{1,2} Additionally, three **DeRoy** derivatives exhibit promising antimicrobial activity and low cytotoxicity.¹ Regarding their mechanism of action, **Roy** appears to target the bacterial cell wall rather than the cell membrane. **Roy** can interact with phospholipid membranes without causing disruption, suggesting its antibacterial effects are mediated through intracellular action.² These findings highlight the potential of these natural compounds as alternatives against resistant bacteria. The current study focuses on evaluating royleanone derivatives against pathogenic bacteria associated with skin conditions, while simultaneously preserving the integrity of commensal skin microbiota to maintain the natural balance of the skin's microbial ecosystem. An in-house library of approximately 40 royleanone derivatives has been developed from lead compounds **Roy** and **DeRoy**. Current efforts involve standard antimicrobial evaluation, including MIC, MBC, and biofilm inhibition assays. Preliminary results have identified four derivatives with strong activity against MRSA and low cytotoxic. This study has the potential to contribute significantly to the development of innovative dermatological therapies targeting conditions associated with microbiota dysbiosis.

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Modulation of bacterial Carbonic Anhydrases: a case study in MedChem

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Ciprofloxacin (CPX) is one of the most employed antibiotics in clinics to date. However, the rise of drug-resistant bacteria is dramatically impairing its efficacy, especially against life-threatening pathogens, such as *Pseudomonas aeruginosa*. This Gram-negative bacterium is an opportunistic pathogen, often infecting immuno-compromised patients with severe or fatal outcomes. The evidence of the possibility of exploiting Carbonic Anhydrase enzymes as pharmacological targets^{1,2} along with their role in *P. aeruginosa* virulence inspired the derivatization of CPX with peculiar CA-inhibiting chemotypes, i.e., benzenesulfonamide and coumarin. Thus, a large library of CPX derivatives was prepared and tested on a panel of bacterial CAs and the human (h) isoenzymes, allowing the establishment of structure-activity relationships (SARs). Computational and crystallographic studies helped to assess the binding pose of the compounds within the CA catalytic site. Importantly, promising preliminary ADME properties in vitro were found, and no cytotoxicity was detected for some representative compounds.³

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Comparison of protective efficacy of two DNA vaccines against *Klebsiella pneumoniae* virulence factors

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Background: *Klebsiella pneumoniae* represents an important clinical problem in the community and hospital settings given the increase of infections caused by hypervirulent and resistant to multiple antibiotics strains, respectively¹. The development of vaccines against this species may help to prevent infections and reduce the associated mortality and morbidity². A promising approach would be DNA vaccines given their stability and flexibility. The aim of this study is to develop two DNA vaccines using *K. pneumoniae* virulence factors belonging to fimbriae and porins, confirm their expression in eukaryotic cell culture, and evaluate the vaccines' immunogenic capacity and protective efficacy against infection.

Material and Methods: Suitability of Fim and Por proteins as antigens was analysed considering their exposure on the outer membrane, their role during infection stage, their essential cellular function and their amino acid sequence conservation between *K. pneumoniae* strains. The sequences of the selected proteins were adapted for expression in humans, fused to immunostimulatory elements, and cloned independently on the DNA platform pVAX1. The resulting vaccine candidates were validated by transfection into HEK-293T eukaryotic cell culture and antigen expression was assessed by Western blot. Vaccines were tested *in vivo* by an immunization assay in mice to analyse antibody levels elicited, and efficacy of protection against sepsis was recorded.

Results: pVAX1-Fim and pVAX1-Por were successfully constructed and expressed both by cellular and supernatant fraction of HEK-293T cells. Significant total IgG, IgG1 and IgG2a subtypes were obtained with both vaccine candidates. pVAX1-Fim was the best candidate to protect against *K. pneumoniae* infection (p=0.0373).

Conclusion: The fimbrial subunit tested can elicit better protection against *K. pneumoniae* infection compared to the porin selected as antigen. DNA platforms can be a good tool for the preclinical development of vaccines against multidrug-resistant bacteria.

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Spatiotemporal Distribution and Diversity of Airborne Resistant Bacteria: An Exploratory One Health Study in the Urban and Rural Environments of Bangladesh

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Antimicrobial resistance (AMR) is a widespread One health issue with planetary impacts. However, there is dearth of knowledge and scientific evidence on the magnitude of resistant bacteria in air and their transmission pathway. Therefore, an exploratory observational study in Bangladesh was conducted to quantify the clinically significant drug resistant bacteria in air with their spatial diversity. This study employed the collection of air samples from both urban and rural settings in four distinct environments – i) Urban live bird markets (LBM) ii) Urban residential area (URA) iii) Commercial poultry farms (CPF) and iv) Rural households (RHH). MacConkey agar supplemented with 3rd generation cephalosporin (3GC) and meropenem respectively was used to obtain 3GC resistant (3GCr) and carbapenem resistant *Enterobacteriaceae* (CRE). Mannitol Salt agar supplemented with oxacillin and Slanetz-Bartley medium supplemented with vancomycin were utilized to obtain Methicillin (Oxacillin) resistant *Staphylococci* (MRS) and Vancomycin resistant *Enterococci* (VRE). The bacterial identification and susceptibility testing were conducted by VITEK 2 system. The presence of 3GCr, CRE, MRS and VRE in 85%, 60%, 100% and 80% air samples was observed respectively. 3GCr, CRE and MRS were highest in CPFs and VRE in LBMs. The abundance (>90%) of MRS, VRE and 3GCr in URA is alarming whereas the air samples from RHHs were heavily burdened with 3GCr and MRS (60-100%). The CRE in poultry environment also establishes the threat added by current farm practice. The diversity and richness of resistant organisms were measured by Shannon diversity index, which was higher in both seasons at LBMs and CPFs (H-2.17-2.21 and H-1.99-2.03 respectively). Considering the organism family, the major bacteria were *Staphylococcaceae* (35%), *Pseudomonadaceae* (20%), *Enterobacteriaceae* (15%), *Moraxellaceae* (10%), *Lactobacillaceae* (7%) and *Enterococcaceae* (6%). This study findings emphasize on the inclusion of air in the system approach and surveillance to tackle AMR due to its high potential for acting as both reservoir and medium of spread of resistance.

The Role of HMGB1 Gene Variations in Diagnosing Sepsis in Newborns Admitted to the NICU

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Introduction: Neonatal sepsis is a critical condition that poses a significant threat to newborns within the first 28 days of life. Diagnosing sepsis in neonates is challenging due to the absence of specific biomarkers, necessitating a comprehensive evaluation of clinical and laboratory indicators. [1,2] The high mobility group box 1 (HMGB1) protein, known for its pivotal role in both metabolic and immune responses, has recently garnered attention in the context of autoimmune and inflammatory diseases.[3] Studies have highlighted the interaction between HMGB1 and AMP-activated protein kinase (AMPK), a key regulator of cellular stress and metabolic processes. Inhibition of HMGB1 has been shown to activate AMPK, suggesting that HMGB1 may play a crucial role in the pathogenesis of sepsis.[4]

Material and methods: This study explored the role of variations in the HMGB1 gene sequence in diagnosing sepsis in newborns admitted to the Neonatal Intensive Care Unit (NICU) with suspected sepsis. A cohort of 69 newborns (51 infants with clinical sepsis and 18 newborns with congenital pneumonia), all suspected of sepsis, was included in the study. The genetic analysis, including whole gene sequencing of HMGB1, was conducted using next-generation sequencing (NGS) at the Genetic Laboratory of the Military Medical Department of the State Security Service of Azerbaijan. Additionally, levels of HMGB1 and AMPK proteins were measured in the blood samples. Sepsis diagnosis was established based on the EMA scoring system, leading to the classification of 51 infants as having clinical sepsis (Group I) and 18 newborns as having congenital pneumonia (Group II). Statistical analysis of the data was carried out using SPSS20 software.

Results: The study revealed a higher frequency of single nucleotide variant (SNV) changes in the sepsis group, with 37.3% of infants exhibiting four SNV changes. Specifically, 52.9% of infants in Group I and 27.8% in Group II ($p=0.066$) displayed SNVs in the HMGB1 gene. Intronic variants such as rs3742305 & COSV58104338 and rs2249825 were consistent across 22 newborns, with one infant exhibiting only one of these variants. The relative risk (RR) for sepsis development due to these SNVs was calculated to be 3.7 for rs3742305 & COSV58104338, 3.57 for rs41376448, and 0.7 for rs1060348, indicating a threefold increase in the risk of sepsis progression associated with these genetic variations. Moreover, the homozygous form of these SNV changes was observed exclusively in infants with sepsis. No significant correlation was found between SNV polymorphisms and procalcitonin (PCT) levels and C-reactive protein (CRP). However, AMPK and HMGB1 levels were notably lower in the sepsis group on the second day of life compared to the pneumonia group. In septic infants, the mean HMGB1 level was recorded at 1510 ± 411 pg/ml, and the mean AMPK level was 1132 ± 401 pg/ml.

In conclusion, the findings suggest that HMGB1 gene variations and AMPK levels may serve as valuable diagnostic markers for neonatal sepsis. Further research is warranted to confirm these associations and explore their potential in clinical practice.

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Do Antibiotic Metabolites have a Role in the Development of Antibiotic Resistance?

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Antibiotic metabolites are produced during metabolism in humans and animals, and they often have very similar structures to the parent antibiotics, with some still possessing antibacterial activity. Antibiotics and their metabolites can be present in wastewater treatment plants, agricultural runoff, and hospital effluent, and from there they can enter the environment.[1] Exposure to sub-inhibitory concentrations of antibiotics can promote the development of resistance in bacteria.[2] However, the impact of bacterial exposure to sub-inhibitory amounts of antibiotic metabolites in the development of resistance to the parent compounds is less clear.

We have adapted and improved literature methods for the synthesis of the main human metabolites of commonly prescribed antibiotics from three different antibiotic classes, namely Ciprofloxacin, Amoxicillin, and Sulfamethoxazole; very commonly used antibiotics which have been detected in the environment previously. We have synthesised these compounds in quantities sufficient for a wide range of microbiological studies. To date, we have conducted sub-inhibitory concentration studies with two metabolites of ciprofloxacin, using the clinically relevant ESKAPE pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and the results of these studies will be presented.

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New Nitrogen-Containing Flavonoids Suppress Gentamicin Resistance of *Staphylococcus aureus*

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Flavonoids have garnered significant attention as naturally occurring compounds due to their diverse biological activities including potent MDR inhibitory activity and generally low toxicity. Previously reported synthetic and naturally occurring nitrogen-containing flavonoids exhibited interesting biological activities [1, 2]. In this study, we synthesized novel derivatives of quercetin and luteolin through Buchwald–Hartwig amination, introducing substituted anilines at the C-8 position [3]. Our investigation revealed that while the aniline modifications abrogated anti-inflammatory activity and maintained low cytotoxicity, they significantly enhanced the flavonoids' ability to combat MDR. Notably, 8-(4-(trifluoromethyl)anilino) quercetin demonstrated potent inhibition of erythromycin resistance in *Staphylococcus aureus* by targeting the ribosomal methyltransferase *ErmA* gene and interacting with its RNA-binding pocket. Additionally, 4-fluoroanilino derivatives effectively suppressed staphylococcal efflux systems, reducing drug resistance at lower concentrations than conventional inhibitors. All derivatives exhibited superior modulation of gentamicin resistance compared to the parent compounds, highlighting their potential in combating MDR. Our findings emphasize the promise of aniline-modified flavonoids as adjuvant therapies to overcome bacterial resistance mechanisms, laying the groundwork for future therapeutic development. Further studies are needed to explore the specificity and broader applicability of these compounds across diverse bacterial genera.

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Lactones as Antimicrobial Agents: From Synthesis to Evaluation

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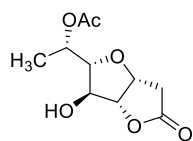
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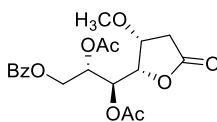
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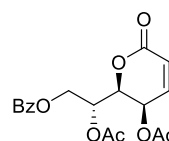
There are a large number of natural products and synthetic analogues that have a lactone ring in their structure. This structural fragment is often responsible for biological activity. The activities discovered so far are exceptionally diverse and potent. In this study, we report a series of different lactones and their antimicrobial activities. These compounds were obtained through various synthetic routes. The main difference in the structure of these compounds is in their lactone moiety (e.g., 5-membered ring, 6-membered ring, single ring, bicyclic system, etc.). The preliminary antimicrobial activity of these compounds was determined as the minimum inhibitory concentration (MIC). Our research aims to find novel antimicrobial agents such as natural products and their synthetic compounds. Our group is willing to contribute to the design and synthesis of compounds and participate in interesting antimicrobial research.



(+)-protulactone A



(-)-cleistanolate



(-)-cleistenolide

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Exploring 5-membered ring spiro p53 activator compounds as antibacterial agents

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In the last years, several bacterial pathogens have been found to disable the key tumour suppressor protein p53 during infection. This disruption hinders the host cell's protective response to the DNA damage that frequently arises from bacterial infections. Thus, this type of infections can drive tumorigenesis by inducing genomic instability, escaping immune surveillance, and modifying the tumour microenvironment.^{1,2} Moreover, several pathogens have been related to promote p53 degradation by activating MDM2.^{2,3} These findings highlight the importance of further exploring the relationship between host-microbe interactions and p53 regulation pathway, as this could reveal new therapeutic targets. Spiro-indolinones and spiro-pyrazolines are considered privileged structures in medicinal chemistry, exhibiting diverse pharmacological activities, including antimicrobial and anticancer.⁴ In this study, we report the synthesis of spirooxindole derivatives⁵ (Figure 1) and the evaluation of their antimicrobial efficacy against reference and clinical strains of *Acinetobacter baumannii*, *Escherichia coli*, and *Pseudomonas aeruginosa* in the absence and presence of colistin. Microdilution assay analysis showed that some of these derivatives have MICs ranging from <0.125 to 32 mg/L against reference and clinical strains of *A. baumannii* and *E. coli* when combined with colistin. These data indicate that spirooxindole derivatives possess antibacterial activity against Gram-negative bacilli, suggesting that they may expand the repertoire of drug treatments against bacteria.

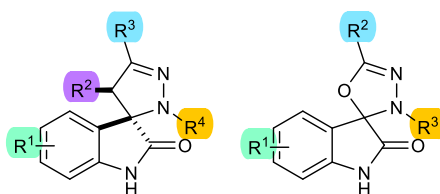


Figure 1. Spiropyrazoline (left) and Spirooxadiazoline (right) core structure of the synthesized derivatives.

Acknowledgement

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DEfeat Antimicrobial Resistance through Iron StArvation in *Staphylococcus aureus* (ERASE): how biochemistry can support the search of novel antimicrobials.

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Iron is a vital nutrient for *Staphylococcus aureus* and other pathogenic microorganisms for survival and infection settlement. To limit bacterial growth, the host organism employs a defense mechanism known as nutritional immunity, which restricts the availability of essential trace minerals. In response to host's iron-limiting defenses, *S. aureus* deploys various iron acquisition systems, including siderophores and the Iron-regulated surface determinant (Isd) [1]. Siderophores are small molecules with a high affinity for iron, secreted to capture ferric iron from host proteins. Among them, staphyloferrin B (SB) is produced by the most virulent coagulase-positive *S. aureus* strains [2]. On the other hand, Isd system works in concert with hemolysins and is in charge to bind free hemoglobin and gain hemic iron. As a first step, the cell-wall anchored receptor IsdB extracts heme from hemoglobin (Hb) and promotes its internalization [3].

The primary aim of ERASE is to lay the bases for a combined, novel approach for developing antimicrobial therapies by identifying 1) ligands that can disrupt the key protein-protein interaction between Hb and IsdB, and 2) inhibitors of the enzyme SbnA, which catalyzes one of the key steps in SB biosynthesis [4]. To pursue these goals, we applied biochemical techniques to support computational, biophysical and microbiological research and gain functional and structural details disclosing *S. aureus* iron acquisition mechanisms and allowing their impairment.

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Membrane-Active AlkylGuanidino Ureas Against Bacteria: An Intriguing Tale

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Antimicrobial resistance poses a serious threat to Public Health, undermining the effectiveness of current antibacterial treatments and highlighting the urgent need for new therapeutic options. We have recently identified AlkylGuanidino Ureas (AGUs, **Figure 1**) as promising membrane-active, broad-spectrum antibacterial agents with minimal inhibitory concentrations (MICs) ranging from 0.12 to 16 $\mu\text{g}/\text{mL}$ and exhibiting bactericidal behavior. What began as a serendipitous discovery¹ evolved through rational design and in-depth biological investigation, providing valuable insights into this chemical class.^{2,3} Thus, a straightforward orthogonal protecting group strategy enabled the generation of new AGU derivatives, expanding our structure-activity relationship (SAR) data. The membrane-based mechanism of action was further elucidated through molecular dynamics (MD) simulations on modeled bacterial membranes. In addition, the entire library of over 70 compounds was tested in combination with subinhibitory concentrations of colistin, a last-resort antibiotic, against colistin-resistant strains to evaluate potential synergistic or additive effects. Finally, cheminformatics and machine learning analyses were used to better understand the critical features contributing to bioactivity.⁴

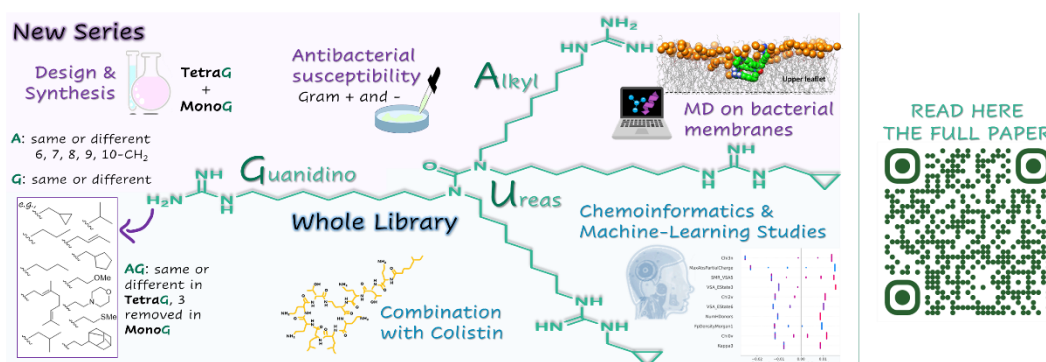


Figure 1. Overview of the work with the chemical structure of a representative AGU compound.

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C2-Linked Arabinose-Functionalized Polystyrene Microbeads Selectively Target *Staphylococcus aureus*

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Staphylococcus aureus is a Gram-positive bacterium, classified as one of the “ESKAPE” pathogens that pose a significant risk to human health through emergence of multi-drug-resistant strains.^[1] Current diagnostic techniques for *S. aureus* are often slow and/or require expensive equipment, highlighting the pressing need for rapid, operationally simple, and cost-effective methods for *S. aureus* detection at the point of care.^[2]

D-arabinofuranose is a rare sugar, found as a key component of the mycobacterial cell wall as well as in the pili of several *Pseudomonas* species.^[3a,b] Additionally, it has been shown that analogues of D-arabinose are metabolised by *Mycobacterium smegmatis* and are uptaken by *Escherichia coli* ^[3a,c]. Therefore, D-arabinose was of interest to our group as a selective targeting motif in bacterial diagnostics. We investigated the use of D-arabinose in a rapid bacterial detection assay based on carbohydrate-functionalized polystyrene microspheres (**Fig. 1**). A library of arabinose derivatives conjugated to microbeads was synthesized, varying in functionalization site and linker length, and subsequently used in agglutination assays. These experiments reveal selective binding of C-2 linked arabinose moieties to *S. aureus*, over a panel of common bacterial pathogens.^[4] The interaction is observed *via* clustering of microbeads after incubation with bacteria, due to multivalent binding between the surface arabinose and *S. aureus*. Further experiments revealed the ability of C-2 linked arabinose microbeads to disrupt *S. aureus* biofilms *via* this interaction.

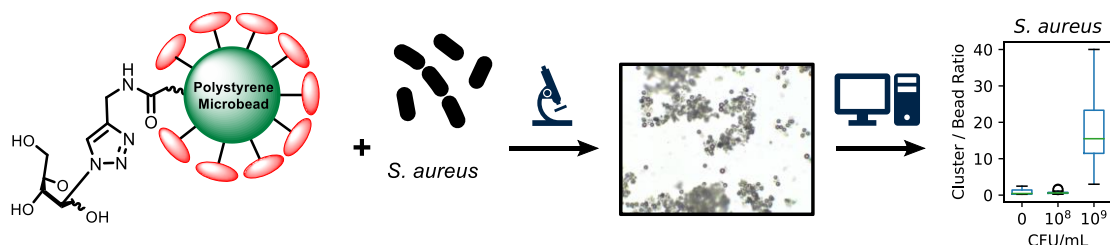


Figure 1: D-Arabinose functionalized microbead agglutination assay allowing detection of *S. aureus*.

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Occurrence and molecular characteristics of cephalosporin-resistant *Escherichia coli* isolated from goose farms in Poland

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Antimicrobial resistance (AMR), especially resistance to third-generation cephalosporins, has emerged globally in food-producing animals, including poultry [1]. Compared to other poultry species, studies of geese are not covered by EU control programs [2]. Thus we aimed to evaluate the prevalence and AMR of cephalosporin-resistant *Escherichia (E.) coli* isolated from goose flocks.

The sampling of 295 goose farms in 2020 and 2022 was proportionally distributed according to the regional density of goose production across Poland. Standardized methods from official AMR monitoring programs were applied to assess the prevalence and AMR status of cephalosporin-resistant *E. coli*. Suspected isolates were subjected to antimicrobial susceptibility testing using the microbroth dilution method (Sensititre; TREK Diagnostic Systems, Thermo Fisher Scientific, EUVSEC3, EUVSEC2). Interpretation criteria were based on epidemiological cut-off values (ECOFF). Whole Genome Sequencing (WGS) was conducted using the NextSeq platform (2x150 bp; Illumina). The sequences were analyzed for AMR genes using Abricate, plasmid replicons using PlasmidFinder 2.1, and multilocus sequence types (ST) using MLST 2.0.

Cephalosporin-resistant *E. coli* were isolated from 33,5% (n=99) goose flocks. All these isolates were resistant to ampicillin and cefotaxime. Above 90% of isolates were also resistant to ceftazidime, cefepime, and ciprofloxacin, and more than 50% resistant to nalidixic acid, tetracycline, trimethoprim, sulfamethoxazole, and chloramphenicol. The WGS results stated a large diversity of genes responsible for ESBL and ampC mechanism of resistance including *bla*_{CTX-M-27}, *bla*_{CTX-M-15}, *bla*_{CTX-M-65}, *bla*_{CTX-M-55}, *bla*_{CTX-M-14} or *bla*_{CMY-2} being the most often. All isolates resistant to colistin possessed the *mcr-1.1* gene. Numerous STs of *E. coli* were identified with ST533 being the most prevalent and correlated with the presence of *bla*_{CTX-M-27}. Among plasmid replicons, IncFIB, IncFII, IncFIA, and IncX1 were the most common.

Our study shows that geese constitute a meaningful reservoir of cephalosporin-resistant *E. coli* carrying multiple resistance determinants including those of public health concern. These findings underscore the need for further surveillance and control measures for AMR in geese.

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DRUG-RESISTANT BACTERIA FROM HONEY JAR, DO YOU WANT TO EAT THEM?

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Paenibacillus larvae, gram-positive, spore-forming bacterium, is globally present and recognized as causative agent of American foulbrood disease in honey bees (*Apis mellifera*). *Paenibacillus larvae* serves as an ideal carrier of resistant genes. Practice of metaphylaxis, aimed at suppressing clinical symptoms of diseased honeybee brood, is a poor management strategy. While it temporarily improves symptoms, it fails to eradicate infection that persists locally in contaminated areas, exhibiting bio-stability similar to other spore-forming *Bacillus* species. Repeated administration of selected antibiotics within hives over extended periods leads to genetic adaptation in bacteria. This results in acquisition of resistance genes against certain antibiotics, compromising disease “control” and cause resistant bacteria in honey.

Contrary to common understanding, *Paenibacillus larvae* is not considered a zoonotic agent. However, in cases of intravenous honey administration, spores could act as infectious agents. In modern times, antimicrobial resistance (AMR) in *Paenibacillus larvae* has been documented, but it receives little attention from medical professionals and their patients.

This communication emphasizes urgent need for well-argued research to clarify resistance mechanisms of *Paenibacillus larvae* and its potential to infect humans, particularly in cases of compromised gastrointestinal integrity or altered microbiomes. Interaction between gut leakage pathology and exposure to unusual AMR bacteria requires detailed investigation. This highlights need for interdisciplinary collaboration to trace potential global pressures from superbugs. While risks of AMR are widely acknowledged, there remains **insufficient focus on non-clinical matrices**, such as those identified within One Health framework. Presence of AMR bacteria in honey—a raw food consumed directly or used in apitherapy—constitutes a significant risk. This concern is amplified by dynamics of global food trade and variability of honey production practices. In some regions, use of antibiotics in beekeeping remains unregulated, often abused, and applied constantly in hives. This not only raises concerns about residual antibiotic levels but also enables flow of resistance genes.

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Abundance of Antibiotic Resistant Genes (ARGs) in Wastewater and Receiving Surface Water

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The occurrence and spread of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) have become an important public health and environmental issue worldwide. During the last decades, an increased rates of antimicrobial resistance has been recognized worldwide, and an increased frequency of multidrug-resistant (MDR) isolates in the clinical setting has been demonstrated, which can be horizontally transferred to human-associated bacteria pathogens through water and food and thus contribute to antibiotic resistant. Climate conditions may influence the abundance and concentration of ARGs in wastewater and surface water. Types and amounts of antibiotics in use may reflect the levels and species of ARGs in wastewater and surface waters. Furthermore, monitoring the types and levels of ARGs by wastewater based epidemiology tools may reflect the amount and types of antibiotic used by the community. This study was conducted to evaluate the role of wastewater treatment plants in the environmental transmission of ARB and ARGs. A wastewater treatment plant and the receiving stream served as the study sites. The levels of fecal contamination in the stream waters were determined by enumerating fecal coliform and the indicator for ARBs was Extended Spectrum Beta-Lactamase (ESBL)-*E. coli*. The abundance of ARGs was determined by the detection of *tetA*, *Int1* and *sul1* and the gene 16s DNA was determined to reflect the total bacteria levels in the samples. The results demonstrate that the level of microbial contaminates in the upstream waters was low and its in agreement with the low detected levels of ARGs. A reduction of 2 logs was recorded for all studied microbial parameters by the secondary biological treatment. Similar reduction was recorded for ARGs in the secondary treatment reactor. The level of fecal coliform, ESBL-*E. coli* and somatic coliphages was not reduced in the two downstream sites moreover, the levels of ARGs were higher in the downstream sites than those observed in the secondary effluent. The high levels of study contaminants in the outfall site of the Yarkon stream indicate that none point pollution is discharged into the stream. The high levels of ARGs present in surface water may facilitate the HGT to pathogenic bacteria. The results of the study indicate that high levels of microbial contaminants and ARGs are discharged to the Mediterranean Sea, which may pose public health and environmental risks.

Multidrug resistance in the lab and *in vivo*: The growing role of 3D Cell Culture in identifying commercially valuable compounds.

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Testing bacteria for drug resistance *in vitro* or in animal models does not make sense. Testing bacteria for drug resistance in humans is not ethical. In December 2022, the FDA has opened the door to resolve this conundrum and signed into law the 'Modernisation Act 2.0'

The use of antibiotics has selected multiple drug resistant strains which are now abolishing their utility. The problem is that bacteria have different growth properties when grown in cultures, and different host preferences in animals compared to when they give rise to pathological conditions in human disease¹. Therefore, to find new antibiotics, the FDA will now accept data from 3D cell culture models which more accurately reflect the *in vivo* human condition.

3D cell culture is one of the most rapidly expanding areas in medical science and 3D models exist for many human tissues. Using them, it is possible to study the importance of genetic diversity as well as *per oral*/ intravenous/ intramuscular compound administration. Numerous models for infectious diseases have already been developed². These encompass a wide variety of microorganisms from unicellular eukaryotes (e.g. plasmodium/ malaria³); yeast (candida/ periodontitis); bacteria (mycoplasma/ pneumonia⁴) and viruses (JEV/ encephalitis⁵). Even more are on the way.

Simultaneously as the successes are being realised, the pitfalls that exist in these models are also coming to light. This allow future testing to be refined and even better *in vivo*-mimicking models to be developed.

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Quaternized phthalocyanines as a powerful tool against a broad spectrum of pathogenic microorganisms and melanoma

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Photodynamic therapy is a promising solution to two major medical challenges: antibiotic-resistant infections and cancer. This study explores the potential of novel cationic phthalocyanines as photosensitizers, focusing on their antimicrobial and anticancer properties. Functionalized with pyridyloxy substituents and bulky moieties like tert-butylphenyl or pyrenyl groups, these compounds exhibited exceptional efficacy against a broad spectrum of pathogens, including *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Trichophyton mentagrophytes*. Microbial viability reductions ranged from 1.4 to 5.6 logs, even at low concentrations, and strong synergy with doxycycline was observed. The photosensitizers demonstrated high singlet oxygen generation, with the anionic counterpart (SO₄²⁻ versus I⁻) significantly influencing efficiency. Photodecomposition studies confirmed moderate stability. In parallel, the anticancer activity of the compounds was tested against melanoma cells, with the most effective derivative reducing cell viability by 85% within one hour. Mechanistic studies highlighted early apoptosis as the predominant cell death pathway. These findings designate the studied phthalocyanines as promising dual-action agents for photodynamic therapy, offering potential breakthroughs in combating antibiotic resistance and treating aggressive cancers like melanoma.

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Rheology and Thermodynamics of Condensates from Ionic Polysaccharides

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Hyaluronic acid and chitosan are semi-flexible biopolyelectrolytes with applications in biomaterials due to their biocompatibility and biodegradability. Complex coacervates of the hyaluronic acid/chitosan system [1] has recently been used as a scaffold of stem cells for cartilage tissue engineering [2, 3]. Here, we will present the effect of various physicochemical properties (pH, ionic strength, molecular weight of polymers) on thermodynamics of HA/CHI coacervates by isothermal titration calorimetry [4]. We will also demonstrate our rheology results which indicate the importance of molecular weight of HA and the degree of acetylation of CHI [5] on viscoelastic properties of the HA/CHI coacervates. Lastly, we will show how HA/CHI coacervates can be converted into chemical gels by modification of these polymers with catechol groups.

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Adjuvant therapy - an effective tool to address antibiotic resistance

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The longest used adjuvant therapy for the treatment of drug-resistant bacterial infections is Augmentin, which has been used in clinical practice for more than 40 years. However, all subsequent adjuvant therapies are also limited to preventing inactivation of β -lactam antibiotics. Inhibitors of other resistance enzymes are so far restricted to scientific publications or preclinical trials.

The goal of our laboratory is to find new inhibitors that could serve as adjuvants for combination therapy of antibiotic-resistant infections. For this purpose, we use clinical isolates of resistant strains, nanopore sequencing, genetic engineering methods to prepare genetically modified bacteria, and subsequent high-throughput screening.

A novel adjuvant of selenoester structure, which effectively inhibits aminoglycoside destructase AAC(6')/APH(2'') of staphylococci and thus enhances the effect of tobramycin, will be presented at the congress. This destructase is highly prevalent in *S. aureus*, including MRSA, and *E. faecalis* and represents one of the most clinically important aminoglycoside-modifying enzyme. It is involved in resistance to gentamicin, kanamycin, tobramycin, netilmicin and amikacin, which it inactivates by phosphorylation and/or acetylation. AAC activity is mediated by the N-terminal domain, while the C-terminal domain is involved in APH activity, and both domains can function independently. AAC(6')/APH(2'') is the only enzyme identified so far that causes gentamicin resistance in *S. aureus* due to phosphorylation (1).

Since the presented adjuvant is a substrate of efflux pumps in Gram-negative bacteria, its application increases the efficacy of aminoglycosides also in *Pseudomonas aeruginosa*, *Acinetobacter baumannii* or *Klebsiella pneumoniae*. This new adjuvant thus offers broad-spectrum use in combination with aminoglycoside antibiotics to treat infections caused by resistant strains.

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POSTERS

Microbiological Contamination of Bed Linen

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Hospital linen is clearly recognized as a potential reservoir for microorganisms and could be a vector of disease transmission. Various species of microorganisms, including some relevant for HAIs, have been isolated from hospital textiles [1, 2]. The aim of the present study was to isolate bacteria from different locations of clean and dirty linen in a surgery department and determine antimicrobial resistance. Microbiological samples have been collected on bed linen. The first group consisted of samples taken randomly from clean bed linen (n=60), before patients lay down for scheduled or emergency surgery. The second group consisted of samples taken from the patient's own used bedding (n=60) immediately after he/s left the surgery department. The samples were taken using sterile cotton swabs. From each bed, 3 samples were taken from different locations: pillow, blanket and sheet. The obtained samples were cultured aerobically on selective media. Antimicrobial susceptibility was determined by discs' diffusion method. The p value of $p < 0.05$ was considered to be significant. In clean bedding, the microorganisms were isolated by 25 and in used bedding - by 65 percent cases. In order to find out whether the microorganisms increase statistically significantly in used bedding, the McNemar test was performed, which showed that $p < 0.05$. This means that clean bed linen can be contaminated at some point before patients use it. The obtained results showed that out of 60 used linen samples, most microorganisms were isolated from contaminated (86.7%) and dirty (61.9%) surgical wounds. We found that mixed microflora (*Staphylococcus epidermidis* and bacteria of the genus *Bacillus*) grew only in bed linen with a contaminated surgical wound. *Candida albicans*, *Pseudomonas aeruginosa* and mixed microflora (*P. aeruginosa* and *S. epidermidis*) grew exclusively in bedding with a clean surgical wound. The results showed that the prevalence of microorganisms slightly different in bedding locations. From this, we can conclude that microorganisms were equally common in different areas of bedding, both in clean and used bedding. The highest resistance was determined for the antimicrobial - cefaclor (94.4%), while the lowest - for ciprofloxacin (44.4%). After studying the resistance of *P. aeruginosa* to antimicrobials it was found that half of the microorganisms are sensitive to ciprofloxacin. *P. aeruginosa* was resistant to all other antimicrobial agents 100 %. This only confirms that the pathogens in the hospital environment have high resistance to the antimicrobials commonly used in the surgical department and are one of the biggest problems these days. During the study, the pathogens of hospital infection were identified: *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Candida albicans*. We found microbial growth on clean bedding that had recently been prepared, brought from the laundry and laid out. This means that clean bed linen can be contaminated at some point before patients use it. We isolated these microorganisms from several samples, but they are among the most common microorganisms causing HI infections. Most opportunistic pathogens isolated from the samples correspond to usual members of human skin microflora or to bacteria from the environment. Since it appears that laundry becomes contaminated after washing, actively antimicrobial textiles would be a valuable tool to prevent textiles from becoming a vehicle for the transmission of microorganisms.

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Innovative Polymyxin Derivatives Targeting Gram-Negative and Gram-Positive Pathogens

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Propolis, a resinous substance collected by bees from plant exudates, has gained recognition for its potent antimicrobial properties. This study investigates the antimicrobial efficacy of propolis sourced from three distinct locations in Latvia, focusing on its activity against a range of Gram-positive and Gram-negative bacteria, clinical isolates, and fungi. Propolis was extracted using six different solvents — acetonitrile (MeCN), ethanol (EtOH), methyl tert-butyl ether (MTBE), dichloromethane (DCM), ethyl acetate (EtOAc), and hexane (HeX) — to explore solvent-specific activity profiles. The extracts and raw propolis were tested against *Escherichia coli* (EC) ATCC 25922, extended-spectrum beta-lactamase-producing *E. coli* (ESBL), *Staphylococcus aureus* (SA) ATCC 25923, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* (PA) ATCC 27853 and *Candida albicans* (CA) ATCC 10231. Antimicrobial activity was assessed through well-diffusion assays, measuring zones of inhibition, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests were conducted to evaluate the potency of the extracts. Additionally, biofilm inhibitory properties were examined using biofilm assays. Results demonstrated that all extracts exhibited notable antimicrobial effects, particularly against Gram-positive bacteria (SA and MRSA), Gram-negative bacteria (PA) and CA fungi. All extracts showcased the most significant activity against CA. Polar solvent extracts (EtOH, MeCN and EtOAc) showed the most significant activity in the well-diffusion and MIC assays, suggesting superior extraction of bioactive compounds. While MTBE and DCM extracts also displayed strong antimicrobial effects. As biofilm formation poses a major challenge in chronic and drug-resistant infections, all extracts effectively disrupted biofilm formation by SA, MRSA, and CA. This finding highlights the potential of propolis as a source of biofilm-inhibitory agents. Although Gram-negative bacteria (EC, ESBL, and Pa) exhibited greater resistance to the extracts, some partial inhibitory effects were noted, indicating a need for further optimization of extraction protocols. This study underscores the broad-spectrum antimicrobial activity of propolis and its extracts, particularly against multidrug-resistant pathogens such as MRSA and CA. The varying effectiveness of the extracts emphasizes the importance of solvent selection in maximizing the recovery of bioactive compounds and providing valuable insights for future research aimed at developing propolis-based antimicrobial therapies. Further research is warranted to identify the specific bioactive compounds responsible for the observed effects and to elucidate their mechanisms of action. Propolis extracts hold promise as natural, sustainable alternatives to synthetic antibiotics, especially in the fight against biofilm-associated infections and multidrug-resistant pathogens.

Lack of RNase HI reduces virulence of *Salmonella enterica*

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Bacterial virulence and antibiotic resistance are interconnected global threats that can synergize, multiplying their adverse effects on health and economy¹. Two proposed strategies to address this dual challenge are non-biocidal inhibition of virulence traits (anti-virulence strategy)² and manipulation of eco-evolutionary dynamics in bacterial populations to hamper dissemination of antibiotic resistance (anti-resistance strategy)³. Design and implementation of these strategies require to identify factors involved in bacterial gene expression, fitness and evolution. R-loops are RNA-DNA hybrids generated during transcription⁴ that strongly influence these traits^{5–8}, as well as fitness of bacteria carrying antibiotic resistance mutations⁹. The enzyme responsible for degradation of R-loops, the RNase HI¹⁰, is thus a potential target for anti-virulence and anti-resistance approaches. Indeed, lack of RNase HI drives antibiotic-resistant bacteria to extinction in populations with high initial frequency of resistance, both *ex vivo* and during colonization of the murine gut¹⁶. Here we investigate the effects of RNase HI deficiency on pathogenicity of *Salmonella enterica*.

We observed that lack of RNase HI alters expression levels of virulence genes, both at population and single-cell levels, and causes defects in motility and biofilm formation. Moreover, we showed that the absence of RNase HI reduces the ability of *Salmonella* to survive within mammalian cells, in agreement with the recently reported exacerbation of bacterial R-loops during intracellular growth¹¹.

Altogether, our results suggest that RNase HI could also serve as a target for anti-virulence strategies.

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Antimicrobial and general toxicity bioactivities of 1,2,4-triazolium hemi-synthetic derivatives

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1,2,3-Triazoles are widely used in the fields of pesticides and medicines due to their broad-spectrum bioactive properties. The synthesis of a new triazole-containing conjugates has gained interest in the past few years. However, these are associated with drug resistance, giving rise to the need to develop novel drugs with similar properties. In this regard, 1,2,4-triazolium appears significant due to its antibacterial, antifungal, anticancer, and antiprotozoal activities¹.

Some of the triazolium compounds, such as Biapenem and Cresamba, have been approved by the U. S. Food and Drug Administration (FDA) as marketed human drugs². Therefore, we set out to synthesize different 1,2,4-triazolium compounds using 1,4-dimethyl-4H-1,2,4-triazolium iodide as the precursor. The synthesized triazolium molecules were evaluated for their antimicrobial, and general toxicity. These were tested against Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and yeast (*Saccharomyces cerevisiae* and *Candida albicans*) strains. Derivatives 1,4-Dimethyl-4H-1,2,4-triazolium iodide and 1,4-Dimethyl-4H-1,2,4-triazolium silver nitrate had the highest antimicrobial activity against both Gram-negative and positive bacteria and yeast with minimum inhibitory concentrations (MICs) values between 0.98 to 15.63 µg/mL. We thus identified which of these 1,2,4-triazolium compounds have the best biological activities and suitable for the synthesis of new lead compounds.

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Aromatic Abietane Diterpenoids as a Promising Scaffold for the Development of ArnT-Mediated Colistin Resistance Inhibitors

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Colistin is a last-resort antibiotic for treating multidrug resistant Gram-negative bacterial infections.¹ In *Pseudomonas aeruginosa*, inhibition of the ArnT transferase (ArnT) is expected to reduce colistin resistance.² Based on this, we have previously identified a natural ent-beyerene diterpene, **FDO**, that reduces colistin resistance and validates the power of the diterpene scaffold as a key platform for the further development of ArnT inhibitors.^{3,4} However, notable issues, such as the low compound concentration in the plant, the need for multistep purification of the raw material, and the absence of chromophore groups, make it challenging to conduct a comprehensive microbiological characterization and develop delivery systems for this compound. Here, we considered rational procedures that allow the simplification of the *ent*-beyerene complex structure into drug-like synthetic molecules to minimize the structures of the pharmacophores that prove to be crucial for biological activity. Therefore, other naturally related diterpene scaffolds were evaluated as a source of starting material to prepare a novel generation of ArnT inhibitors. In particular, this study investigated the aromatic abietane scaffold of podocarpic acid **1**, which lacks the ring D compared to the *ent*-beyerene one, maintains the same configuration at C-4 with respect to **FDO**, and features an aromatic ring C and a hydroxyl group at the C-12 position. Semisynthetic oxygen and nitrogen derivatives of **1** were designed, synthesized, and tested against colistin-resistant *Pseudomonas aeruginosa* strains to exploit the versatility of the aromatic abietane diterpene scaffold. Diterpene **2**, featuring an alcohol group at C16, and its esters derivatives, **3**, **5** and **6**, emerged as strong candidates for further experimental investigation, showing potent inhibition of colistin-resistant strains. The aromatic abietane skeleton was identified for the first time as a privileged scaffold for the further cost-effective development of valuable colistin resistance inhibitors.

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Functional genomics against antibiotic pollution and resistance genes

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Due to their widespread misuse, antibiotics and their resistance genes have become an increasingly concerning problem. They constitute a problem for both human health and the environment. From the health perspective, the WHO estimates that antibiotic multiresistant bacteria are responsible for 700000 deaths per year. On the other hand, antibiotics and their resistance genes have been found to be present in water bodies and soils. To overcome this problem, we propose two main strategies: (i) the use of functional metagenomics to detect the presence of antibiotic resistance genes in different environments, which will help prevent and combat the emergence of resistant pathogens. and (ii) the use of bacterial consortia to biodegrade antibiotics found as pollutants in river waters to reduce the emergence of resistant bacteria and resistance genes.

We have screened a library constructed in our laboratory using metagenomic DNA extracted from a pristine soil from the Alcornocales Natural Park in Cádiz. In this library, we have identified several ofloxacin, norfloxacin and sulfamethoxazole resistance genes. On the other hand, we are currently constructing a new metagenomic library with environmental DNA extracted from the Guadaira River, in Seville, a river with a significant concentration of these antibiotics. Comparison of the number and type of resistance genes found in both environments will provide information on the impact of this contamination on the emergence of resistance genes and resistant bacteria.

At the same time, we are performing serial enrichment cultures with samples from wastewater treatment plants growing in minimal media supplemented with ciprofloxacin, trimethoprim or sulfamethoxazole as sole carbon source.

To date, we are obtaining very promising results, and in the near future, we aim to sequence the metagenomes of the consortia to identify bacterial species which can effectively degrade these pollutants.

Zebrafish as a Model for Bacterial Infections: Advances in Antimicrobial Discovery and Pathogenesis Research

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The rise in the prevalence of antibiotic-resistant bacterial infections represents a major global health challenge. Over the past 30 years, the development of new effective antimicrobials has stalled. One of the main obstacles in the development of new therapies is the discrepancy observed between *in vitro* and *in vivo* trials, which significantly delays the translation of compounds into clinical practice. The mouse has been classically employed in pharmacological studies; however, it is an expensive, complex model that raises ethical concerns. This highlights the need for a more efficient model, such as zebrafish (*Danio rerio*), a vertebrate widely used in genetics and developmental biology research. Its use in the field of infectious diseases began in the 1990s and has increasingly demonstrated its benefits in recent years. Our objective is to evaluate the current utility of zebrafish as a model for human bacterial infections, as well as a platform for screening new antimicrobials, emphasizing its advantages and the latest advancements in the field of microbiology. To this end, a bibliographic search was conducted in databases such as PubMed, Scopus, Web of Science (WOS), and Google Scholar, using the keywords "zebrafish", "bacterial infection", "antimicrobial screening" and "human infection", selecting publications between 2010 and 2024.

Overall, zebrafish offers numerous advantages that make it an ideal model for studying bacterial infection. It is a small-sized animal, easy and inexpensive to maintain. The high availability of embryos and their external development in water have facilitated high-throughput screenings of new antimicrobial compounds, as well as innovative strategies such as phage therapy or the discovery of new virulence factors through signature-tagged mutagenesis. Additionally, zebrafish embryos are transparent during the first days of development, providing significant optical accessibility. This transparency allows *in vivo* host-pathogen interaction studies using high-resolution microscopy with specific fluorescent markers to identify the pathogen and host immune cells. Moreover, zebrafish possesses both innate and adaptive immune systems, with the latter developing four weeks post-fertilization. This unique feature enables the isolated study of the role of innate immunity in the context of infection. Its genome was fully sequenced in 2013, revealing 70% homology with the human genome and 82% of the genes associated with human diseases. Consequently, numerous highly accessible gene-editing techniques have been developed, leading to the discovery of susceptibility polymorphisms to certain infections, later confirmed in patients.

In summary, these advantages are reflected in significant advances in the pathogenesis and treatment of infections caused by *Shigella flexneri*, *Mycobacterium tuberculosis*, *Burkholderia cenocepacia*, *Streptococcus pyogenes*, and *Staphylococcus aureus*, as detailed in this study. These findings exemplify its potential to bridge gaps between preclinical and clinical research. By leveraging its strengths, zebrafish can contribute to accelerating the discovery of effective therapies, addressing the global challenge of antimicrobial resistance.

Searching for new antimicrobials and novel antibiotic resistance genes through functional metagenomics

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The misuse of antibiotics has dramatically increased the prevalence of bacteria resistant to multiple antimicrobial compounds. Combined with the limited discovery and approval of new antibiotics each year, this poses a severe global health problem. While most antibiotics are produced by bacteria, only 1% of them can be cultured under laboratory conditions. To overcome this limitation, we use a functional metagenomics approach that enables DNA expression from any bacteria in a specific environment without culturing them.

Our group has developed vectors and strains to facilitate the heterologous expression of environmental DNA. In our laboratory, we maintain metagenomic libraries containing DNA from various environmental sources.

The screening of these metagenomic libraries through the heterologous expression system has led to the discovery of clones with antimicrobial activity against high-priority pathogens like Methicillin-resistant *Staphylococcus aureus* and other clones that are resistant to hospital-use antibiotics such as meropenem, ciprofloxacin, and tigecycline. Some clones contained known sequences, validating our strategy, while others are particularly interesting as their genes had not been previously associated with these activities.

This metagenomic approach has proven useful, as it has identified known antimicrobial compounds and antibiotic resistance genes while also enabling the discovery of new resistance genes and antimicrobial production genes.

Antibiotic, toxicity, and anti-inflammatory properties of the inhibitors of SARS-CoV-2 main protease

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The pandemic caused by the coronavirus (SARS-CoV-2) has significantly affected global public health. During the pandemic, it has become important to prevent secondary infections and manifestations of inflammation. SARS-CoV-2 main protease (Mpro, also known as 3CLpro) has been identified as one of the most attractive targets within the key enzymes of SARS-CoV-2 for drug design due to high specificity, the conserved structure, and the low toxicity of Mpro inhibitors to host cells. We systematically screened the scientific databases for compounds with activity against SARS-CoV-2 Mpro together with their antibacterial and anti-inflammatory properties. Data were collected using PubMed, Scopus, PubChem, ChEMBL and SciFinder® scientific search databases.

Oral acute toxicity (rodent) was predicted using a virtual lab, ProTox-II (Banerjee et al., 2018) (https://tox-new.charite.de/protox_II/). The predicted values of oral rodent LD₅₀ values were calculated together with the assessment of hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity. SwissAdme platform (Daina et al., 2017) was used for the prediction of physico-chemical and ADME properties. Graphical and statistical evaluations were done with the programs MOE and Statistica (Statistica version 12.0). Structural similarity analysis for 49 compounds using the Fingerprints MACCS Structural Keys and Tanimoto coefficients for similarity metrics has been performed by means of MOE software. Consequently, the cluster distribution for the given compounds was evaluated based upon 75% similarity and 75% overlap.

We identified several compounds proper for the new design, structural enhancement and experimental studies, such as apigenin-7-O-glucoside, GC376, nirmatrelvir, 6-Chloroquinazoline, walrycin B, dipyridamole, bepridil, Z-DQMD-FMK, narlaprevir, tideglusib, carmofur and masitinib.

Complete data collection including antibacterial activities of the inhibitors can be found in <https://doi.org/10.1016/j.tiv.2023.105640>.

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Bacterial DNA methylation plays a role in controlling antibiotic susceptibility

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Infections caused by antibiotic-resistant bacteria pose a major threat to human health worldwide. Therefore, there is an urgent need to find new therapeutic strategies that can solve this global problem. Classical research on antibiotic resistance has attempted to understand how spontaneous mutations in certain target genes or their regulators allow bacteria to acquire resistance to antibiotics and how the exchange of mobile genetic elements between bacteria allows horizontal gene transfer that contributes to the spread of resistance determinants. However, changes in the DNA sequence cannot fully explain either the speed of development of antibiotic resistance or the existence of transient resistance. Therefore, the idea that epigenetic mechanisms could be contributing to antibiotic resistance is increasingly relevant.

We have explored whether DNA methylation in bacteria has a role in controlling antibiotic susceptibility using 414 antibiotic-resistant *Escherichia coli* clinical isolates. These isolates were sequenced by Illumina and Oxford Nanopore Technology in order to analyze their genetic and epigenetic modifications. Susceptibility to various antibiotics, as well as the presence of heteroresistance, was also evaluated in these clinical isolates.

The results derived from this study show an interesting pattern in the frequency of methylases among resistant clinical isolates compared to non-resistant isolates, as well as a relationship between methylation patterns and susceptibility to some antibiotics.

DNA methylation appears to play a relevant role in antibiotic resistance and could inspire the development of new therapeutic strategies to combat antimicrobial resistance.

Antibacterial activity of chalcone derivatives against *Staphylococcus aureus*

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In the present days, the abundant use of antibiotics for human or animal therapy leads to the development of microbial resistance to multiple drugs. As a consequence, the infections by multidrug-resistant (MDR) bacteria represent an increased and severe problem for the global public health.¹ A noted case is the methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant not only to methicillin but usually also to aminoglycosides, macrolides, tetracycline, chloramphenicol, and lincosamides.¹

Chalcones are naturally-occurring compounds, widely used in traditional medicine, with plant extracts to be the most important sources of chalcones.² Their structure, a three-carbon α,β -unsaturated carbonyl moiety joins two aromatic rings, is presented in a variety of biologically active natural and synthetic compounds and is considered as an open chain intermediate in the synthesis of flavones.³ Recently, chalcones and synthetic derivatives showed to be promising agents for combating the multidrug resistance of *S. aureus* to drugs.⁴

An initial screening of 60 chalcones derivatives, provided 4 promising compounds active against the resistant *S. aureus* with MIC values between 1 and 40 μM (Figure 1). Some of these molecules were able to reverse the resistant phenotype when combined with ciprofloxacin, thus the ability to inhibit efflux systems was tested using a semi-automated fluorometric method. Based on these scaffolds, new derivatives were designed and synthesized providing more active molecules. Our goal is to describe the relationship between structure and activity and identify key structural motifs.

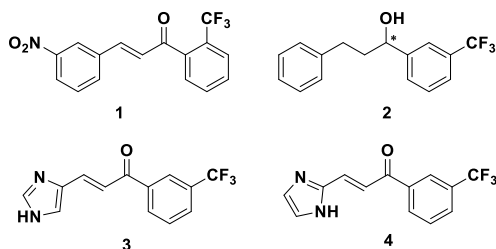


Figure1. Structure of active chalcone derivatives active against *S. aureus*.

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Polyphenolic content, antioxidative and antimicrobial activity of *Eryngium amethystinum* L. methanolic extracts

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Eryngium amethystinum L. is a plant which grows in Balkan and Apennine peninsula. The aerial parts, roots and fruits are used in ethnopharmacology of Italy and Western Balkan countries. Traditional preparations of *E. amethystinum* were used in treatment of oedema, malaria and gastrointestinal diseases. Methanolic extracts of *Eryngium amethystinum* L. leaves, stems and inflorescences were prepared in order to examine its antimicrobial and antioxidative activity and to determine polyphenolic content of prepared extracts. DPPH test was conducted in order to examine antioxidative activity of prepared extracts. Polyphenolic compounds were determined using Folin-Ciocalteu method. Antimicrobial activity was examined using broth microdilution method. The tested microorganisms were *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The highest antioxidative activity was observed in leaf extract of *E.amethystinum*, with observed IC₅₀ value of 21.795 µl/ml. Intermediate value was observed in flower extracts, with IC₅₀ of 36.8816 µl/ml, while stem extract showed the lowest antioxidative activity with IC₅₀ of 57.16 µl/ml. The highest content of polyphenols was observed in leaf extract, with concentration of 0.053171 mg GA/mg of dry extract, intermediate value was observed in extract of inflorescences 0.04078 mg GA/mg of dry extract and the lowest amount was observed in stem extracts 0.011552 mg GA/mg of dry extract. Leaf and flower extracts showed activity against *Staphylococcus aureus* with MIC =2.5 mg/ml. Extracts did not exhibit activity against Gram-negative bacteria and *Candida albicans*. All the observed results show the significance of further investigation of methanolic extracts of *Eryngium amethystinum*, its chemical composition and pharmacological activity.

Key words: *Eryngium*, antimicrobial, antioxidative, polyphenols

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N-Phenylpyrrolamide DNA gyrase inhibitors: Promising antibacterial agents against high-priority pathogens and mycobacteria

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The increasing threat of antibiotic resistance underscores the urgent need for novel antibacterial agents with unique mechanisms of action. The ATP-binding site of DNA gyrase and topoisomerase IV represents a promising antibacterial target. These enzymes share about 40% sequence identity and have similar active sites, making them attractive for dual targeting strategies. Antibiotic-resistant bacterial infections, including mycobacterial infections, pose a major public health challenge. *Mycobacterium tuberculosis* (Mtb) and non-tuberculous mycobacterial infections, such as those caused by *M. abscessus*, are on the rise worldwide, with limited treatment options and increasing drug resistance.

In this study, we investigated the development of *N*-phenylpyrrolamides as inhibitors of DNA gyrase B, focusing on compounds with in vitro activity against "ESKAPE" pathogens and mycobacteria. The most effective compounds showed IC₅₀ values in the low nanomolar range against DNA gyrase and topoisomerase IV. Their minimum inhibitory concentrations (MIC) were in the low micromolar range for both Gram-positive and Gram-negative bacteria. Some of the compounds also showed activity against selected mycobacteria. For the most promising candidates, we investigated the development of resistance, post-antibiotic effects (PAE) and their impact on a *S. aureus* zebrafish model for systemic infections. These results highlight the potential of *N*-phenylpyrrolamides as a promising class of dual-target antibacterial agents with significant activity against resistant pathogens.

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Chitosan-linked antimicrobial peptides promote eradication of *Staphylococcus aureus* biofilms

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Bacterial resistance to antibiotics is a global challenge, with the current clinical pipeline of new antimicrobial drugs remaining insufficient to tackle the problem [1] Thus, the need for development of new modalities of antimicrobial drugs is important and urgent. Antimicrobial peptides (AMPs) are promising compounds for the treatment of bacterial infections, which have been investigated as drug candidates since more than two decades. Advantages of AMPs include fast onset of activity, slower emergence of resistance, broad-spectrum activity against bacteria, fungi, and viruses, and ability to bypass the common resistance mechanisms facing conventional antibiotics [2]. However, challenges such as low selectivity for pathogenic cell surfaces over endogenous human cells leads to serious toxicity issues [3]. It is well-established that most cationic AMPs attain significant antimicrobial activity by interaction of multiple copies directly with the polyanionic microbial cell surfaces to cause membrane disruption [4]. In 2015, we were the first to perform chemical coupling of multiple copies of the AMP anoplin onto a chitosan scaffold and obtained *proof-of-principle* that this approach leads to an enhancement of antimicrobial potency and simultaneously reduces the hemolytic toxicity, providing a dramatic increase in therapeutic index [5]. Chitosan is a biopolymer composed of β -(1-4)-linked D-glucosamine units, and is a natural, biodegradable and nontoxic material. In our current study, we studied four different antimicrobial peptides- anoplin, Hs02, Bac2A, and IDR-1018 that were coupled to three different chitosans (MW 10-40 kDa). All conjugates displayed enhanced activity against *Staphylococcus aureus* (planktonic and biofilm) and dramatic decrease in hemolytic activity as compared to isolated AMPs. This shows the potential of chitosan-AMP conjugates as a platform technology.

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The Role of HMGB1 Gene Variations in Diagnosing Sepsis in Newborns Admitted to the NICU Pusta Orujova PhD, Azerbaijan Medical University, 2nd Child Disease Department

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Introduction: Neonatal sepsis is a critical condition that poses a significant threat to newborns within the first 28 days of life. Diagnosing sepsis in neonates is challenging due to the absence of specific biomarkers, necessitating a comprehensive evaluation of clinical and laboratory indicators.[1,2] Although bacteriological examination is regarded as the gold standard for sepsis diagnosis, it often fails to provide definitive results. The aetiology of sepsis is multifactorial, involving bacterial, viral, and fungal infections; however, the progression to sepsis is modulated by deficiencies in the innate immune system and underlying metabolic disorders. The high mobility group box 1 (HMGB1) protein, known for its pivotal role in both metabolic and immune responses, has recently garnered attention in the context of autoimmune and inflammatory diseases.[3] Studies have highlighted the interaction between HMGB1 and AMP-activated protein kinase (AMPK), a key regulator of cellular stress and metabolic processes. Inhibition of HMGB1 has been shown to activate AMPK, suggesting that HMGB1 may play a crucial role in the pathogenesis of sepsis.[4]

Material and methods: This study aimed to explore the role of variations in the HMGB1 gene sequence in diagnosing sepsis in newborns admitted to the Neonatal Intensive Care Unit (NICU) with suspected sepsis. A cohort of 69 newborns, all suspected of sepsis, was included in the study. These infants were admitted to the NICU of the Scientific-Research Institute of Pediatrics and the Republican Perinatal Center, both operating under the Ministry of Health of the Republic of Azerbaijan. Inclusion criteria for the study encompassed a gestational age between 34 and 42 weeks, clinical suspicion of sepsis, and the acquisition of written parental consent. Routine clinical analyses and instrumental examinations were performed upon admission, and a 2 mL peripheral blood sample was collected in EDTA tubes for genetic analysis. The genetic analysis, including whole gene sequencing of HMGB1, was conducted using next-generation sequencing (NGS) at the Genetic Laboratory of the Military Medical Department of the State Security Service of Azerbaijan. Additionally, levels of HMGB1 and AMPK proteins were measured in the blood samples. Sepsis diagnosis was established based on the EMA scoring system, leading to the classification of 51 infants as having clinical sepsis (Group I) and 18 newborns as having congenital pneumonia (Group II). Statistical analysis of the data was carried out using SPSS20 software.

Results: The study revealed a higher frequency of single nucleotide variant (SNV) changes in the sepsis group, with 37.3% of infants exhibiting four SNV changes. Specifically, 52.9% of infants in Group I and 27.8% in Group II displayed SNVs in the HMGB1 gene. Intronic variants such as rs3742305 & COSV58104338 and rs2249825 were consistent across 22 newborns, with one infant exhibiting only one of these variants. The relative risk (RR) for sepsis development due to these SNVs was calculated to be 3.7 for rs3742305 & COSV58104338, 3.57 for rs41376448, and 0.7 for rs1060348, indicating a threefold increase in the risk of sepsis progression associated with these genetic variations. Moreover, the homozygous form of these SNV changes was observed exclusively in infants with sepsis. No significant correlation was found between SNV polymorphisms and procalcitonin (PCT) levels and C-reactive protein (CRP). However, AMPK and HMGB1 levels were notably lower in the sepsis group on the second day of life compared to the pneumonia group. In septic infants, the mean HMGB1 level was recorded at 1510±411, and the mean AMPK level was 1132±401.

HMGB1 gen variations vè alleles in groups

Groups	Variation exists	Variation not exist	homozygote	heterozygote
I group	27(52,9%)	24(47,1%)	6(11,8%)	45(88,2%)
II group	5(27,8%)	13 (72,2%)	0	18(100%)
Pearson Chi-Square value	3,387 P=0,066		2.319 P=0.128	
Likelihood value	3,497 p=0,061		3.825 p=0.05	
Fisher exact Test	Two-sided p=0,99		Two-sided p=0.328	

	One-sided p=0,057	One-sided p=0.150
Odds Ratio for value	0,342, CI-95% lower -0,106, upper-1,100	
For cohort sepsis	Value=0.769, CI 95% lower-0.581, upper-1.017	0.714, CI-95% lower -0,611,upper-0.835
For cohort pneumonia	Value=2.249, CI 95% lower 0.899, upper 5,623	

In conclusion, the findings suggest that HMGB1 gene variations and AMPK levels may serve as valuable diagnostic markers for neonatal sepsis. Further research is warranted to confirm these associations and explore their potential in clinical practice.

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Importance of single cell analysis in the study of antibiotic resistance

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Antibiotic resistance is a global health problem. Current therapies do not take into account subpopulations capable of surviving antibiotic and heteroresistant clinical isolates are increasingly relevant. Thus, a deeper understanding of the phenotypic heterogeneity of antibiotic-resistant bacteria after antibiotic exposure and the transitions between these phenotypes is essential to develop effective infection control strategies.

In this work, flow cytometry was utilized to investigate the behaviour of an isogenic *Salmonella enterica* culture exposed to antibiotics at the single-cell level. Exponentially growing cultures were treated with carbapenems (ertapenem) and fluoroquinolones (levofloxacin) for 4 hours. The expression of different genes involved in antibiotic resistance was analyzed after antibiotic exposure, enabling the study of phenotypic heterogeneity within the isogenic population.

Four different families of genes involved in antibiotic resistance are studied in this work: porins, efflux pumps, and genes involved in cellular stress response and cell envelope synthesis. Distinct subpopulations with varying expression levels of these resistance genes emerged within the isogenic culture. Ertapenem treatment induced subpopulations with differential expression of porins and genes linked to stress response and cell envelope synthesis. In contrast, its effect on efflux pumps was limited, causing only a slight increase in their expression. Levofloxacin exposure, however, led to greater variability in the expression of stress response and cell envelope genes. Additionally, it significantly altered the expression of certain porins and efflux pumps.

Exposure to fluoroquinolones leads to increased efflux pump AcrA expression and reduced porin expression, along with enhanced phenotypic heterogeneity in cell envelope synthesis genes. Conversely, carbapenems induce greater phenotypic heterogeneity in porin and cellular stress response gene expression, while also increasing the expression of genes involved in cell envelope formation.

The observed phenotypic variability may contribute to antibiotic treatment failure in clinical settings.

Unraveling the influence of DNA methylation on antibiotic resistance

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Antibiotic resistance represents a significant challenge to global public health. Traditional approaches have focused on the role of genetic mechanisms; nevertheless, these mechanisms do not fully explain the resistance phenomenon. In fact, recent research highlight that the study of non-mutational genetic mechanisms, such as DNA methylation, could reveal novel therapeutic targets that might help in the fight against antibiotic resistance. The main objective of this study is to investigate the potential involvement of DNA methylation in antibiotic resistance in *Escherichia coli*.

Copy number of the methyltransferase genes *dam*, *dcm*, *yhdJ*, and *hsdM* was investigated in *E. coli* genomes available at the BioSample database from the National Center for Biotechnology Information (NCBI). The analysis was performed in both sensitive and antibiotic resistant clinical isolates. As control, copy number of genes reported to be involved in antibiotic resistance (*ompC* and *groEL*), as well as housekeeping genes (*adk*, *mdh*, and *icd*), not related to resistance, were also monitored in these isolates. To further assess the possible role of DNA methylation in antibiotic resistance, growth of *E. coli* *dam*, *dcm*, *yhdJ*, and *hsdM* mutants was tested in presence of antibiotics.

Antibiotic resistant isolates exhibit greater number of copies of methyltransferase genes than sensitive isolates. This pattern is more pronounced in multidrug resistant isolates (resistant to more than 10 antibiotics) than in isolates resistant to 1 or 2 antibiotics. Our result show that isolates resistant to gentamicin and tobramycin aminoglycosides present more copies of *dam*, *dcm*, *yhdJ*, and *hsdM* genes than sensitive isolates. Accordingly, the copy number of *ompC* and *groEL* genes, both related to antibiotic resistance, also varies between sensitive and resistant isolates, while the number of the housekeeping genes *adk*, *mdh*, and *icd*, not associated with antibiotic resistance, remains consistent between sensitive and resistant isolates. *E. coli* mutants lacking DNA methylation, present retarded growth in presence of some aminoglycosides, thus suggesting the potential implication of DNA methylation in the resistance to these drugs.

Our findings suggest that DNA methylation carried out by Dam, Dcm, YhdJ, and HsdM may be involved in the resistance of *E. coli* to aminoglycosides. Thereby, DNA methylation could be considered as a potential target against antibiotic resistance.

Innovative Polymyxin Derivatives Targeting Gram-Negative and Gram-Positive Pathogens

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Antimicrobial resistance is a major global health threat, with which the antibiotic discovery pipeline is not keeping up. Polymyxins (PM) B and E have resurrected as last-resort drugs (given their toxicity), especially against Gram-negative bacterial infections. However, PM resistance has already been reported, resulting in the lack of effective therapies. Taking advantage of these valuable scaffolds, we set out to design new PM analogs with high antimicrobial activity and low toxicity. α,α -Dialkylglycines (DAG) are unnatural amino acids with unique characteristics (bioactivity, metabolic resistance, hydrophobicity, stability), making them innovative building blocks in PM analog design. We report the synthesis of 6 new PM analogs and their antimicrobial (including against Gram-negative and Gram-positive bacteria) and anti-biofilm activities, as well as investigate their synergy with common antibiotics.

The Ugi multicomponent reaction was applied to the synthesis of 2 DAGs, diisobutyl glycine (Dibg) and dibenzyl glycine (Dbng), which were assembled in situ at positions 6 and/or 7 of native PMB/E (Figure1) through microwave-assisted solid-phase peptide synthesis. The anti-bacterial activities were assessed by broth microdilution (MIC; MBC) against 24-hour cultures of *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and an MRSA strain. Their anti-biofilm activity was then evaluated in *P. aeruginosa* by counting the culturable biofilm cells. The synergy between PM analogs and two common antibiotics - ciprofloxacin (CIP) and tobramycin (TOB) - was evaluated through checkerboard assays and anti-biofilm activity of the PM-antibiotic combinations by CFU counting. The cytotoxicity was evaluated in lung epithelial cells (A459) by the MTS viability assay.

Analog PMBa and PMEa (Dibg @ position 7) were the most promising against *P. aeruginosa*, with PMBa exhibiting MIC/MBC=4 mg/L and no toxicity (A549 lung epithelial cells). Preliminary HPLC analysis indicates ~66% purity, meaning pure analog concentrations ~2 mg/L, close to that of PMB (1 mg/L). Furthermore, PMBa and PMEb (Dibg @ position 6) analogs showed great inhibitory effects in Gram-positive bacterial cultures, with MIC values reduced 16-fold for *S. aureus* and 4-fold for MRSA. Concerning antibiofilm activity, the PMBa analog at 512 mg/L (~340 mg/L pure analog) decreased biofilm ~3.7 log(CFU/mL), which is promising considering that PMB achieved a similar reduction, ~3.9 log(CFU/mL), for the concentration range 128-512 mg/L. In combination scenarios, analogs PMBb and PMEb (Dbng @ position 6) and PMBc (Dbng @ position 6 + Dibg @ position 7) achieved synergic/additive outcomes ($0.3125 < FICI < 0.75$) combined with TOB/CIP, despite biofilm eradication assays showing indifferent outcomes.

We successfully developed 6 PM analogs, 5 exhibiting anti-pseudomonal activity and 2 showing promising activity against *Staphylococci* spp. Analogues were also promising in combination scenarios, revealing DAGs' suitability for PM analog design.

A Way to Immobilization of Antimicrobial Peptides on PDMS Surface

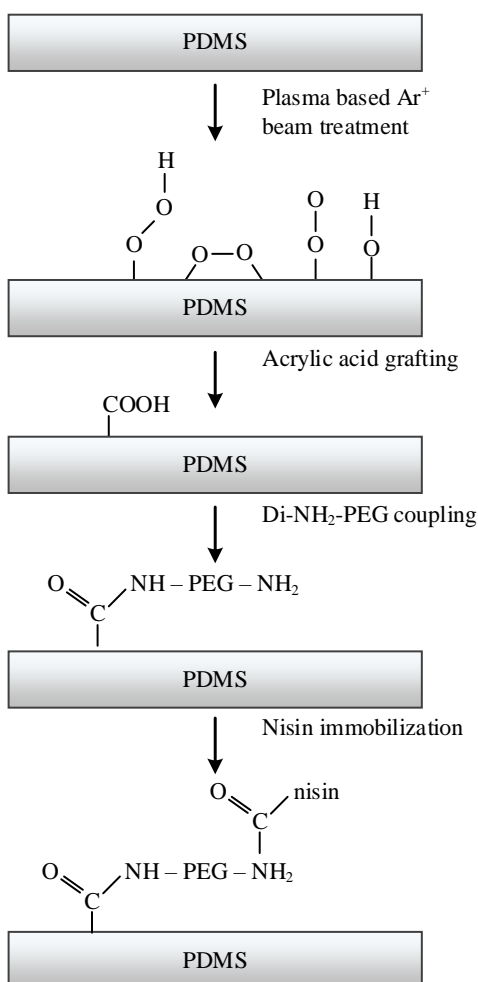
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The aim of this investigation is to demonstrate the ability of plasma based Ar⁺ beam (PBAIB) to initiate immobilization of antimicrobial peptides onto the chemically inert PDMS surface, using the bacteriocin nisin as an example. Earlier developed by us multi-step procedure was



utilized that makes possible three types bonding of antimicrobial peptides: i) linker – free, at the first step, just after the PBAIB treatment; ii) via vinyl monomer linker, at the second step after grafting of vinyl monomer and; iii) via flexible spacer after coupling of di-NH₂PEG on vinyl monomer grafted surface (Fig. 1). A parallel plate reactor, equipped with a serial capacitance, was employed to ensure arise of an ion flow inside the plasma volume. The successful immobilisation of nisin via flexible spacer (di-NH₂-PEG5000) was proved by XPS analysis. This multi-step procedure has a potential to be used whenever need arises to control antimicrobial activity of PDMS or other chemically inert polymeric materials and medical devices fabricated by them.

Fig. 1 Scheme of antimicrobial peptides immobilization, initiated by plasma based Ar⁺ beam treatment (PBAIB)

Targeting Bacteria-Induced p53 Suppression in Cancer with tryptophanol-derived isoindolinones

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Growing evidence highlights the critical role of bacteria in the development and progression of cancer, although the precise molecular mechanisms underlying this interaction remain poorly understood. Certain cancer-associated bacteria secrete toxins that disrupt the host's tumour-suppressive defences, often targeting key regulatory proteins, such as p53. These bacterial toxins may contribute to tumorigenesis by promoting genomic instability, evading immune surveillance, and altering the tumour microenvironment.^{1,2} Additionally, as DNA damage and reactive oxygen species (ROS) are potent inducers of p53-mediated apoptosis, bacterial pathogens have evolved diverse strategies to circumvent p53 signalling. These findings underscore the need for further investigation into the complex interplay between host-microbe interactions and p53 regulation, which may unveil novel therapeutic targets for cancer prevention and treatment.³

Enantiopure tryptophanol-derived isoindolinones were previously identified by our research group as direct reactivators of wt-p53 and mut p53, and some derivatives showed p53-dependent *in vivo* antitumor activity.⁴ In this communication we will disclose the synthesis of a small series of enantiopure tryptophanol-derived isoindolinones, as well as their antimicrobial efficacy in the absence and in the presence of colistin against reference strains of *Acinetobacter baumannii*, *Escherichia coli* and *Pseudomonas aeruginosa*.

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Harnessing the MICROBE interactive visualization tool to unveil trends of antimicrobial resistance burden across Europe (1990–2021)

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Antimicrobial resistance (AMR) is a growing threat to global health, with significant variation across different regions of Europe. The MICROBE (Measuring Infectious Causes and Resistance Outcomes for Burden Estimation) visualization tool provides a transformative platform to explore AMR burden across geographies, age groups and pathogen-drug combinations over three decades (1990–2021). Leveraging data from 520 million individual records and 19,513 study-location-years, this tool integrates information from surveillance systems, hospital databases and systematic reviews to enable comprehensive, interactive analyses. The tool supports dynamic visualizations of AMR burden through user-friendly features such as trend analyses, age-specific breakdowns, geographic heatmaps, as well as comparisons of fatal and nonfatal health outcomes. It reveals critical patterns, such as a decline in AMR-related mortality among children under five years, juxtaposed with a significant increase in mortality among those aged 70 years and older. The tool also highlights disparities in resistance trends, with methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Klebsiella pneumoniae* emerging as key contributors to mortality across European regions. MICROBE’s dual counterfactual approach quantifies deaths attributable to AMR (resistant pathogens replaced by susceptible ones) and deaths associated with AMR (resistant infections eliminated entirely), providing actionable insights. Additionally, users can explore the burden of novel pathogen-drug combinations, resistance trends over time, as well as the fraction of resistance in the overall infectious burden. By enabling policymakers, researchers and public health professionals to identify hotspots, the MICROBE tool is an invaluable resource in the fight against AMR. The MICROBE viz tool can be accessed here: <https://vizhub.healthdata.org/microbe/>

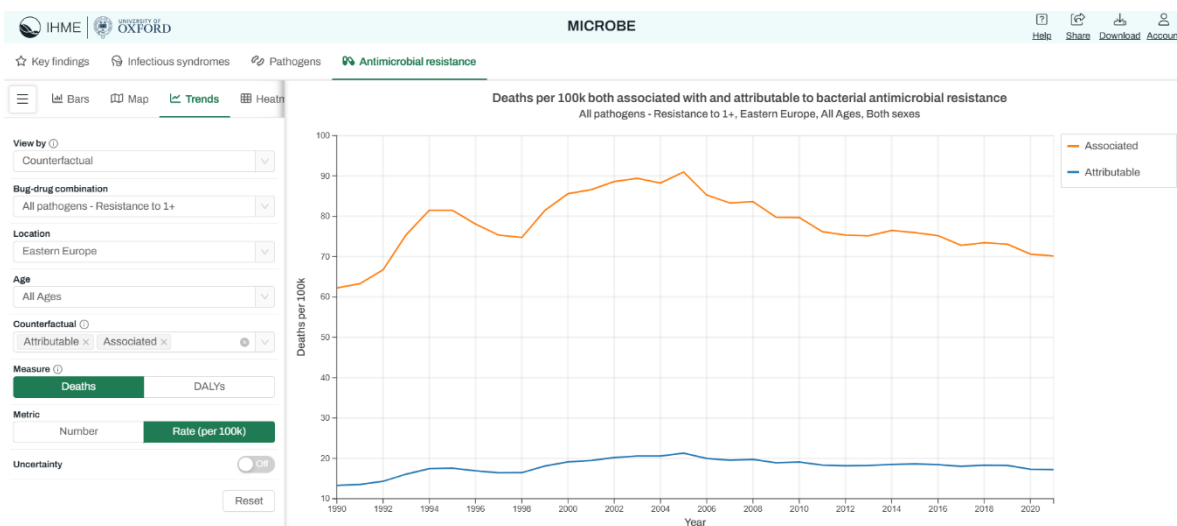


Figure 1. Deaths associated with and attributable to bacterial antimicrobial resistance per 100,000 in Eastern Europe, from 1990 to 2021. Source: MICROBE (Measuring Infectious Causes and Resistance Outcomes for Burden Estimation) interactive visualization tool for exploring estimates of the burden of infections classified by the involved organ system (infectious syndrome), causative microorganism (pathogen), and resistance to treatment (antimicrobial resistance). Link: <https://vizhub.healthdata.org/microbe/>