

EURESTOP WG meeting

4 July 2024

Brussels, COST Association, 23rd floor of the Manhattan
Center, Avenue du Boulevard – Bolwerklaan 21, 1210 Brussels
Belgium

Scientific Committee:

Mattia Mori (Italy) - Action Chair

Patricia Rijo (Portugal) - Vice Chair

Cristina Nativi (Italy) - Science Communication coordinator

Priyanka Sahariah (Iceland) - Grant Awarding coordinator

Dana Reichmann (Israel) - WG1 Leader

Younes Smani (Spain) - WG2 Leader

Carole Devaux (Luxembourg) - WG3 Leader

Tomislav MESTROVIC (Croatia) - Stakeholders Coordinator


Didem SEN KARAMAN (Turkey) - Young Researchers & Innovators Coordinator

Stephen HAWSER (Switzerland) - Science Strategy Advisor

PRACTICAL INFORMATION FOR MEETINGS AT COST ASSOCIATION: HOW TO GET TO HERE




COST Association is located on the **23rd floor of the Manhattan Center** (next to the Rogier square). The building entrance is between the Delhaize supermarket and Thon Hotel.

 **Address :** Avenue du Boulevard - Bolwerklaan 21, 1210 Brussels – Belgium

 **Address :** Rue des Croisades – Kruisvaartenstraat 19, 1210 Brussels – Belgium

Public parking : 2.80 € / hour – 18€ / 24 hours

 **Metro Station :** Rogier (metro lines 2 & 6 tram 3, 4, 25, & 55)

Transport from airport to COST Association



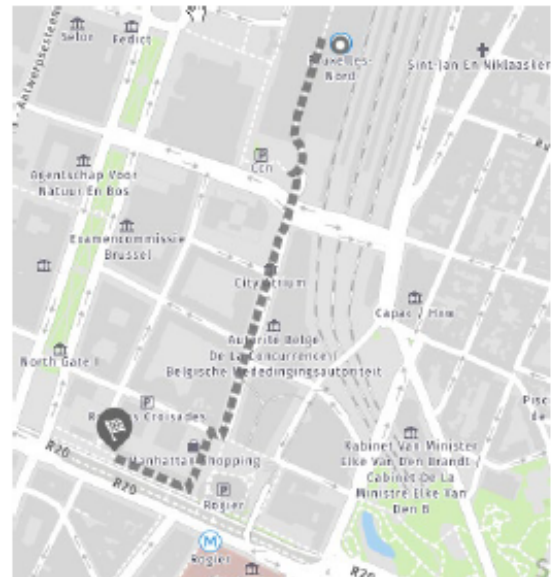
TRAIN:

Brussels North Station (5 minute walk)
Gives direct access from Brussels airport
Frequency: 4 trains per hour
Trip: 15 minutes
Price: 18.60 € return – 9.30 € single (2nd class)



TAXI:

Trip: round 35 to 40 min (morning 8 am and afternoon 5 pm)
Price: 45 € to 50 € (single trip)



AGENDA (room Marie Curie)

Link for remote connection: [CLICK HERE](#) (Meeting ID: 312 154 892 124; Passcode: MLggVd)

- 08:50 – 09:00 **COST Association staff** – Welcome and safety/evacuation instruction.
- 09:00 – 09:15 **Mattia Mori** (Italy, EURESTOP Chair): *EURESTOP updates.*
- 09:15 – 09:30 **Stefano Sabatini**, Italy: *The fight against antimicrobial resistance: our long journey in search of MFS efflux pumps inhibitors.*
- 09:30 – 09:45 **Giorgia Giovannini**, Switzerland: *Responsive nanomaterials to tackle antimicrobial resistance.*
- 09:45 – 10:00 **Luigi Cutarella**, Italy: *Atomistic simulations of bacterial membranes to unravel mechanisms of small molecules internalization.*
- 10:00 – 10:15 **Enkelejda Goci**, Albany: *Antimicrobial evaluation of Carbopol-Capsaicin gel.*
- 10:15 – 10:30 **Martina Fiabane**, Italy: *Unraveling the druggability of bacterial DNA G-Quadruplexes: a study on mexC-G4 from Pseudomonas aeruginosa.*
- 10:30 – 11:00** **Coffee break**
- 11:00 – 11:15 **Spyridon Mourtas**, Greece: *The use of Thiol Chemistry in the Development of Peptide-Based Drugs and Drug Delivery Nano Systems.*
- 11:15 – 11:30 **Silvia Cammarone**, Italy: *Discovery and development of diterpene-based inhibitors of ArnT-mediated colistin resistance.*
- 11:30 – 11:45 **Valentina Puca**, Italy: *Microorganisms Isolated from Patients with Bacteriemia and Study of Antimicrobial Resistance.*
- 11:45 – 12:00 **Simona Bartkova**, Estonia: *Droplet emulsion for probing aggregation and biofilm formation of bacteria.*
- 12:00 – 12:15 **Erika Adomavičiūtė**, Lithuania: *Formation and Investigation of Polymeric Antibacterial Materials.*
- 12:15 – 12:30 **Tanel Tenson**, Estonia: *Antibacterial Compounds Against Nongrowing Bacteria.*
- 12:30 – 13:45** **Lunch & chats**
- 13:45 – 14:00 **Maria Rosa Loffredo**, Italy: *Effect of a single non-proteogenic amino acid on the effectiveness of a frog skin-derived antimicrobial peptides against multidrug resistant infections.*
- 14:00 – 14:15 **Dua Özsoylu**, Germany: *Surface-molecularly imprinted polymer-based biosensor for the detection of pathogenic bacteria.*
- 14:15 – 14:30 **Jonathan Gómez-Raja**, Spain: *The EU-JAMRAI-2 project: Joint Action Antimicrobial Resistance and Healthcare-Associated Infections 2.*
- 14:30 – 14:45 **Ursula Bilitewski**, Germany: *Compound logistics for EURESTOP.*
- 14:45 – 15:00 **Iglika Lessigiarska**, Bulgaria: *In-silico studies of Mycobacterium tuberculosis DNA-gyrase complexes and estimation of potential anti-tuberculosis activity of natural products from Rubia species*
- 15:00 – 15:05 **Demokrat Nuha**, Turkey: *Synthesis of Phthalazine Derivatives: Biological Evaluation and Molecular Insights for Alzheimer's Disease*
- 15:05 – 15:30** **Coffee break**
- 15:30 – 17:00 **Round Table** – *Enhancing WG1 activities: discussion on proposals by WG1 Leader and the Core Group* (moderator: Dana Reichmann).
- 17:00 – 17:10 Closing remarks and next events/grants/activities

SCIENTIFIC ABSTRACTS (in order of presentation)

The fight against antimicrobial resistance: our long journey in search of MFS efflux pumps inhibitors

Giada Cernicchi,^a Tommaso Felicetti,^a Andrea Astolfi,^a Maria Letizia Barreca,^a Violetta Cecchetti and **Stefano Sabatini^a**

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The problem of antibiotic resistance among pathogenic bacteria has reached crisis proportions. Treatment options for infections caused by multi-resistant bacteria are gradually diminishing. The current rate of discovery of new antibacterial agents lags behind the rate of development of new resistance. Efflux pumps play a central role in making a bacterium resistant to multiple antibiotics because of their ability to expel a wide range of structurally diverse compounds. In addition to evading antibacterial compounds, efflux pumps are also involved in bacterial stress response, virulence, quorum sensing, biofilm formation and alteration of host physiology. Efflux pumps are unique and challenging targets for the discovery of novel efflux pump inhibitors (EPIs). EPIs could help rejuvenate our currently depleted antibacterial drug discovery pipeline.

The Major Facilitator Superfamily (MFS), the largest family of transporters, contributes to bacterial survival, adaptation and pathogenicity through the export of diverse molecules. The functions of several relevant MFS multidrug efflux pumps in human life-threatening bacterial pathogens such as *S. aureus*, *L. monocytogenes*, *K. pneumoniae*, *Shigella/E. coli*, *A. baumannii* have been described.

Despite a low degree of sequence identity, MFS transporters share a conserved structural organization, termed the "MFS fold", and a common transport mechanism involving alternate access of the substrate binding site to the cytoplasmic and periplasmic/extracellular sides of the membrane, with the transporter cycling between inwardly open, occluded and outwardly open conformations.¹

This lecture will describe our long-lasting journey in the search for potent MFS EPIs, from the target selection to the early efforts conducted in the search for *S. aureus* NorA EPIs.² Subsequently, thanks to the collaboration with worldwide experts, I will present the translation of acquired knowledge to the inhibition of other MFS efflux pumps of *S. aureus* (QacA) as well as of microorganisms such as nontuberculous mycobacteria (NTM, *M. smegmatis* and *M. avium*),³ *Rhodococcus equi*,⁴ and *S. pseudintermedius*⁵.

References

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Responsive nanomaterials to tackle antimicrobial resistance

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Bacterial infections remain one of the most concerning health problems worldwide, causing millions of deaths and hospitalizations each year. In recent years, much effort has been invested in the development of new methods guaranteeing an early and specific diagnosis to treat infections promptly and selectively. This is of particular importance given the ease with which bacteria can spread and their capability to develop resistance against antibiotics. Different detection techniques have been developed to reduce the detection time and avoid cultivation and sample enrichment, which are particularly time-consuming and could take between 24–48 hours.

In this presentation, I will present stimuli-responsive nanomaterials specifically designed to improve the diagnosis of bacterial infections, with the final aim of improving their treatment and limiting the spread of antimicrobial resistance. In some cases, fluorescent nanomaterials are proposed as point-of-care tools enabling the rapid diagnosis of infections from human specimens[1]. Alternatively, the nanomaterials are specifically designed to enable their incorporation into biocompatible hydrogels and thus achieve wearable sensors for the diagnosis of infected wounds[2,3]. In particular, I will focus on fluorescent-based indirect approaches to sensitively and selectively detect bacteria (i.e. *K. pneumoniae*, *S. aureus*), showing the results achieved with specifically designed nanomaterials enabling the cost-efficient indirect detection of bacteria. Finally, I will present the preliminary data obtained with wearable self-care systems in which the rationale of the recently funded projects with which we aim to combine the diagnosis and treatment of infected wounds.

References

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ATOMISTIC SIMULATIONS OF BACTERIAL MEMBRANES TO UNRAVEL MECHANISMS OF SMALL MOLECULES INTERNALIZATION

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Antimicrobial resistance (AMR) poses a serious threat to global public health in the 21st century. The escalating need to develop new antimicrobial treatments capable of successfully addressing multi-resistant pathogens has prompted the scientific community to explore innovative approaches to combat AMR. The bacterial cell membrane has emerged as a key molecular component for the efficacy of drugs, and in the genesis of antibiotic resistance, gaining significant interest as a potential target for the development of novel antimicrobials.

The complexity of gram-positive and gram-negative bacterial membranes poses a challenge at the molecular level. In particular, the outer layer in gram-negative bacteria represents an additional degree of complexity compared to gram-positive bacteria. Recently, the use of computational modeling has unveiled fundamental aspects regarding the structure and organization of bacterial cell membranes. Through atomistic molecular dynamics (MD) simulations, in this study we aim to elucidate the interaction and internalization process of small molecules into bacterial membranes. Specifically, we focused our efforts on the interaction of the adarotene derivatives drug and the membrane of *S. aureus*. The same approach based on MD simulations was used to explore the internalization of colistin into the membrane of *P. Aeruginosa* may vary depending on its protonation state. Both inquiries can provide valuable insights into key interactions between small molecules and bacteria membranes, thereby contributing to study the interaction between drugs with bacterial membranes.

Antimicrobial evaluation of Carbopol-Capsaicin gel

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This study investigates the antimicrobial efficacy of a phytopharmaceutical Carbopol-based formulation containing capsaicin. Capsaicin, a bioactive compound derived from chili peppers, is known for its analgesic and anti-inflammatory properties. The formulation was tested against a spectrum of microbial strains, including Gram-positive and Gram-negative bacteria, and fungi. Standard antimicrobial assays, such as the broth microdilution method and minimum inhibitory concentration (MIC) tests, were employed to determine the formulation's effectiveness. Results indicated that the Carbopol-capsaicin formulation exhibited significant antimicrobial activity, with notable zones of inhibition and low MIC values against several pathogens. The synergistic effect of Carbopol and capsaicin enhances the formulation's potential as a therapeutic agent for topical infections. These findings suggest that this novel formulation could be an effective option for treating dermatological conditions with an infectious component.

Keywords

Antimicrobial, Carbopol, Capsaicin, Dermatological Formulation

References

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Unraveling the druggability of bacterial DNA G-Quadruplexes: a study on mexC-G4 from *Pseudomonas aeruginosa*

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Antimicrobial resistance (AMR) poses a serious global threat to public health, compromising the effectiveness of infection prevention and treatment due to the emergence of drug-resistant pathogens. The lack of new and effective antibiotics underscores the urgent need for new therapeutic targets with low propensity to induce drug resistance. DNA G-quadruplexes (G4s) are emerging as promising targets for their prevalence in crucial genomic regions such as promoters. The advent of Next-Generation Sequencing (NGS) has facilitated rapid acquisition of genomic sequences, bolstering the exploration of G4s as potential therapeutic targets. Despite extensive studies in human contexts, their potential as therapeutic targets in bacteria remains largely underexplored. [1]

In this study, we focus on the G4 identified in the *mexC* gene, which is related to the Resistance-Nodulation-Division (RND) efflux pumps of *Pseudomonas aeruginosa*. [2] The 3D structure of mexC G4 was roughly modeled on deposited PDB structures, and then validated by molecular dynamics (MD) simulations. A library of natural compounds was screened against mexC G4 by molecular docking simulations, resulting in a compound that was investigated by biophysical studies together with known G4 ligands such as TMPyP4, BRACO-19, RHPS4, and PDS.

Finally, biophysical studies such as thermal shift assay (TSA) and circular dichroism (CD) were carried out to assess the compound-induced stability of the mexC G4 and coupled with extended MD simulations to provide a structural hypothesis on the binding of small molecules to the mexC G4.

[1] ACS Omega. 2024 May 30;9(23):24163-24180

The use of Thiol Chemistry in the Development of Peptide-Based Drugs and Drug Delivery Nano Systems

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Sulphur plays an important role in many biological processes and therefore many pharmaceuticals contain this atom. Therefore, the development of methods for the synthesis of thiol-containing compounds and the introduction of sulfur atom into peptide sequences is a useful approach towards the development of thiol-containing peptide-based drugs. To this end we developed: (a) methods for the synthesis of suitably protected mercapto acids, aminothiols, thiol-containing amino acid derivatives and building blocks of interest, and (b) solid-phase synthesis methodologies based on trityl-type resins for the introduction of the synthesized thiol-containing compounds into peptide chains, by the synthesis of methylenethio isosters, thiol-containing peptoids, and of 2-benzothiazolyl-containing amino acids and peptides. In addition, the use of thiol chemistry and our approaches towards the development of targeted drug delivery nano systems are also presented and discussed.

Discovery and development of diterpene-based inhibitors of ArnT-mediated colistin resistance

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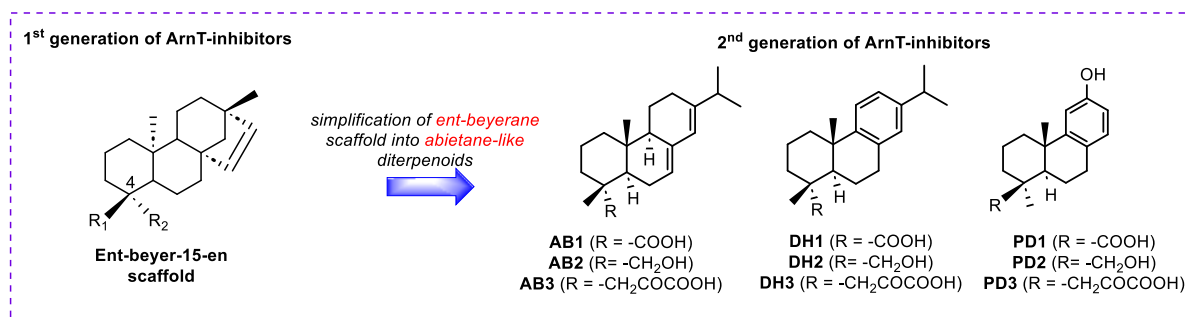
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Colistin is a last-line antibiotic used for the treatment of multidrug resistant Gram-negative bacterial infections. However, a resistance phenomenon due to the glycosyltransferase enzyme ArnT has been recently documented.¹ By combining microbiological assays and molecular modeling, we have previously demonstrated that the diterpene scaffold is a promising platform for the development of novel ArnT inhibitors.² In order to further optimize this scaffold, we set up a rational procedure that simplifies the ent-beyerane scaffold into drug-like synthetic molecules. We have been able to select the abietic, dehydroabietic and podocarpic acid (AB1, DH1, PD1) as profitable starting points for the development and synthesis of a second generation of ArnT inhibitors. Furthermore, with the aim of enlarging the abietane derivatives library, the corresponding hydroxylated derivatives (AB2, DH2, PD2) were synthesized, tested against colistin-resistant *Pseudomonas aeruginosa* strain and their binding mode was investigated through molecular docking simulations. These studies highlighted PD2 as the most promising compound to restore colistin sensitivity in bacteria. Thus, our efforts are focused on the synthesis of new derivatives of PD2 with the aim to investigate the SAR of the podocarpic scaffold.



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Microorganisms Isolated from Patients with Bacteremia and Study of Antimicrobial Resistance

Valentina Puca^a, Benedetta Pellegrini^a, Giorgia Stornelli^a, Asia Libbi^a, Roberta Z. Marulli^b, Pamela Di Giovanni^c, Mattia Mori^d, Piergiuseppe Cantiello^b, Rossella Grande^a

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Bacteraemia, in its strictest sense, refers to the presence of viable bacteria in the bloodstream. Asymptomatic bacteraemia can occur in ordinary activities such as dental practices and after minor clinical procedures and in healthy subjects is transient and does not cause further sequelae [1]. When the immune system fails or is overcome, bacteraemia can evolve into a bloodstream infection (BSI) with clinically relevant sequelae and organ failure and is differentiated as septicaemia [1]. In recent years, the identification of multi-drug resistant bacteria in BSI is becoming very common [2]. The antimicrobial resistance is a global health issue in the treatment of clinical infections, therefore, the aim of this retrospective study was the identification of microbial species responsible of bacteraemia, as well as the assessment of their drug susceptibility pattern.

The present retrospective study has been performed from 2019 to 2023 on blood culture samples collected by the microbiology laboratory of San Pio Hospital, Vasto (Chieti, Italy). The study included 558 patients admitted to different hospital wards, most of them (45%) aged between 60 and 79 years. The microorganisms responsible for the BSI were isolated by culture methods, identified via biochemical tests and analysed for their susceptibility patterns to antimicrobial drugs via the Walk Away automated system. The data showed that the 70.6% were mono-microbial infections whereas the 29.4% were poly-microbial infections. The most common bacterial *genera* detected were *Staphylococcus spp.* (45.3%), *Escherichia spp.* (16.7%), *Klebsiella spp.* (11.3%) and *Enterobacter spp.* (7.2%). We concentrated our attention towards bacteraemia cases sustained by a single bacterial species. We obtained that the 89.8 % of these patients presented at least one mono-drug resistance and the 77.6 % of detected bacteria showed multi-drug resistance. Among bacteraemia cases sustained by a single bacterial species, β -lactam class was the antimicrobial class toward which the bacteria showed the highest percentage of resistance corresponding to 43.1%, followed by aminoglycosides (14.1%), macrolides (8.9%), quinolones (8.3%), lincosamides (2.1%), rifamycins (2%). Limiting the spread of antimicrobial resistance is a public health goal to be pursued and requires the control of multi-drug resistant bacteria via revised therapeutic treatments. This study shows that in most cases, BSI are associated with at least one microorganism resistant at least to one antimicrobial drug. The reported data could help clinicians to establish, based on microbiological analysis, guidelines for the correct choice of a proper therapeutic regimen, in order to treat and manage the BSI and consequently avoiding the outcome of septicaemia.

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Droplet emulsion for probing aggregation and biofilm formation of bacteria

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Antimicrobial resistance (AMR) is a global emergency. Though it is a highly complex issue with many aspects, such as bacteria's adhesive potential, still poorly understood (2). Same and different strains of bacteria can stick together (auto- and co-aggregation respectively) and to surfaces (biofilm formation) (2). Especially biofilm formation facilitated by microplastic (MP) is threatening because plastic pollution is ubiquitous, and MP analysis is problematic (3). We show that our user-friendly droplet emulsion pipeline enables studying multiple facets of AMR, including bacterial autoaggregation and potential MP biofilm formation. We used a poly(dimethylsiloxane) (PDMS) microfluidic chip with flow-focusing geometry to generate monodisperse water-in-oil droplets with encapsulated GFP-labelled *Escherichia coli*. We performed single cell-based (droplet) minimal inhibitory concentration assays for Cefotaxime and Kanamycin. Samples either included only antibiotic solution or antibiotic plus added 10 μm carboxylated polystyrene microspheres. Droplets were incubated for 24h at 37°C, followed by fluorescence imaging of droplet monolayer and analysis via software Ilastik (4) and CellProfilerTM (5). Results indicated that our droplet emulsion pipeline enabled studying potential antibiotic specific *E. coli* susceptibility and autoaggregation (possible early biofilm formation) trends in thousands of replicates.

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Formation and Investigation of Polymeric Antibacterial Materials

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The KTU research group focuses on the development of polymeric materials with enhanced antimicrobial activity without the use of antibiotics. Main research areas:

1. **“Green” synthesis of nanoparticles.** Silver nanoparticles (AgNPs) are synthesized by an eco-friendly and cost-effective method using medical plant extracts as a reducing and capping agents. Secondary metabolites (flavonoids, phenolic compounds, etc.) are responsible for the increase of biosynthesized AgNPs antibacterial, antioxidant, antiinflammatory activity [3].
2. **Hybrid nanocomposites of graphene oxide and silver nanoparticles.** Such hybrid composites are an effective material for controlling nosocomial infections caused by drug-resistant bacterial strains such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. After the hybrid composite come into contact with bacteria, the cell membrane becomes shrivelled, damaged and constituents leak out [2].
3. **Silicone coatings.** A new type of silicone coating with high antimicrobial efficacy has been developed and protected by patent (Patent US11033580B2).
4. **Electrospun antibacterial micro-nano fibre mats.** A technology for antibacterial medical dressings from micro-nanofibres obtained by electrospinning are developed. The compositions are made by using hydrolysates of natural proteins (collagen, keratin) or biocompatible synthetic polymers of high concentration and good spinnability with bioactive additives of plant components and biopolymers. The influence of composition and other parameters on the quality, antioxidant and antimicrobial activity, biocompatibility, cell proliferation, kinetics of release of biologically active compounds, etc. of the nano-microfibres is assessed [1, 4].

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Antibacterial Compounds Against Nongrowing Bacteria

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During chronic infections a large fraction of infecting bacteria are non-growing and escape antibiotic therapy. We screened 6480 registered drugs and drug candidates (Prestwick Library and Specs Drug Repurposing Set) for activity against non-growing uropathogenic *Escherichia coli* (UPEC). Hits were identified by monitoring the delay of bacterial outgrowth after dilution of the treated stationary phase cultures into fresh growth medium¹. The primary screen was performed in diluted CA-MHB medium (pH 7.4) as well as in low phosphate, low magnesium medium (pH 5.5) that mimics conditions in the endocytic compartments. We observed a significant impact of the screening protocol on the results. Notably, Semapimod, the most potent hit discovered in a similar screen that was run in a nutrient supplemented PBS₂, was inactive under our testing conditions. 39 hit compounds of different classes (19 fluoroquinolones, 5 macrolides, 1 pleuromutilin, 4 rifamycins, 7 anti-cancer drugs and 3 antiseptics) were further tested against non-growing UPEC, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Dose-dependent bactericidal activity was characterised by monitoring regrowth delay as well as CFU decrease. 10 compounds (Solithromycin, Rifabutin, Mitomycin C and 7 fluoroquinolones) were strongly bactericidal against non-growing *P. aeruginosa*, killing more than four logs at 2.5 μ M concentration, with a strong effect evident already after a one-hour treatment. An in-depth efficacy comparison of these compounds with the currently used treatments against persistent infections is warranted.

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Effect of a single non-proteogenic amino acid on the effectiveness of a frog skin-derived antimicrobial peptides against multidrug resistant infections

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Cationic alpha-helical antimicrobial peptides (AMPs) are key effectors of the innate immunity and considered as new alternative anti-infective agents to cope with the increasing number of multi-drug resistant microbial infections. The AMP Esculentin-1a (Esc 1-21) (peptide 1), a derivative of the N-terminal portion of a frog-skin AMP, is known for its capacity to inhibit the growth of Gram-negative bacteria, while it weakly affects Gram-positive bacteria [1,2]. In this study, three analogues of peptide 1 were designed by the replacement of a single amino acid with a non-coded residue (peptide 2), or with a Proline (peptide 3) or D-Proline (peptide 4) and investigated for their structure-activity relationship and mechanism of action on both living and model systems. The results indicated that peptide 2 has enhanced activity against Gram-positive bacterial strains, without being cytotoxic to eukaryotic cells. In addition, fluorescence assays on bacteria and large unilamellar vesicles (LUVs) mimicking the microbial cell membranes demonstrated that peptide 2 has a membrane perturbing activity. Nuclear magnetic resonance and circular dichroism spectroscopy were also used to analyse the structure of the most active peptide 2. Our data demonstrated that the better biological activity of peptide 2 compared to the original peptide 1 is the result of a combination of different features, including increased biostability, increased alpha helical content as well as the adoption of a deformed helix with the capacity to decrease the membrane fluidity. Overall, it has been shown how a single amino acid substitution is able to enlarge the spectrum of activity of its parent peptide, thus assisting the optimization of AMPs for the development of new broad-spectrum anti-infective agents.

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Surface-molecularly imprinted polymer-based biosensor for the detection of pathogenic bacteria

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In today's globalized world with a highly mobile and interdependent nature, pathogenic bacteria-related diseases spread rapidly, which causes a serious global threat to modern health care as well as environment and food safety. For instance, according to the estimations of the World Health Organization (WHO), every year, 600 million people become ill after consuming contaminated food (1), where most of them are related to pathogenic bacteria (2), and 420,000 people die every year due to the consumption of contaminated food (1). To combat this global threat, the development of advanced diagnostic methods for the detection and monitoring of pathogenic bacteria is crucial.

Surface-molecularly imprinted polymers (surface-MIPs)-based biosensors show great potential in direct detection of the target bacteria. However, the major drawbacks of the current fabrication method of surface-SIPs arise from the requirement for fresh template bacteria and often non-reproducible bacteria distribution on the stamp substrate. In this work, we developed a positive master stamp containing photolithographic mimics of the template bacteria (e.g., *E. coli* as a model organism) enabling reproducible fabrication of biomimetic surface-MIPs-based biosensors without the need for the "real" bacteria cells (3). It was shown that the presence of the biomimetic *E. coli* imprints with a specifically designed geometry increases the sensor bacteria-capturing ability by an "imprinting factor" of about 3. These findings revealed the importance of geometry-guided physical recognition in bacterial detection. In addition, this imprinting strategy was integrated into interdigitated electrodes and quartz crystal microbalance chips. The sensor's *E. coli* detection performance was demonstrated with electrochemical impedance spectroscopy and quartz crystal microbalance measurements with a dissipation monitoring technique.

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The EU-JAMRAI-2 project: Joint Action Antimicrobial Resistance and Healthcare-Associated Infections 2

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Antimicrobial resistance (AMR), particularly antibiotic resistance, has arisen as a serious cross-border threat to health, potentially undermining modern medicine. In the EU, antibiotic resistance accounts for at least 33,000 deaths, 2.5 million extra hospital days, and an estimated societal cost of about €1.5 billion each year. A global approach is called for to combat AMR, combining awareness raising, surveillance, antimicrobial stewardship (AMS), and infection prevention/control (IPC) while ensuring access to antibiotics and diagnostics. Such interventions need to be grounded in a One Health perspective, considering the interconnection between humans, animals and the environment. Following the 2017 EU One Health Action Plan against AMR to make Europe a best practice region and the first European Joint Action (JA) on AMR and Healthcare-Associated Infections (EU-JAMRAI), this new JA, EU-JAMRAI 2, will support Member States/Associated Countries (MS/AC) in their efforts to develop and update their National Action Plan (NAP) on AMR.

The Extremadura region in Spain is performing different tasks on antimicrobial stewardship in humans, animals and the environment and contributing to raising awareness about this topic by reviewing existing communication campaigns and developing new ones based on the identified gaps.

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Compound logistics for EURESTOP

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The COST-action EURESTOP suggested the collection of small molecules from partners, who are active in organic synthesis or are interested in the development of new approaches to fight infectious diseases. In autumn 2023 research groups were invited to submit purified compounds to establish this unique compound library, which was offered to be hosted at the HZI. On the basis of material transfer agreements (MTAs) until now 16 research groups followed this invitation and sent in total 358 compounds, usually between 1-3 mg of the solid materials. These amounts are sufficient to prepare stock solutions, usually 10 mM in DMSO. They are stored at -80°C until use. As soon as research groups are identified, who want to use these compounds for screening, assay-ready plates are prepared from these stock solutions and sent to the screening group. This procedure minimizes the consumption of the compounds, but requires close interaction between the HZI and the screening group, as information about the assay format is required to transfer suitable amounts of the compounds into the assay plates. Examples of respective workflows will be shown.

***In-silico* studies of *Mycobacterium tuberculosis* DNA-gyrase complexes and estimation of potential anti-tuberculosis activity of natural products from *Rubia* species**

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The antibiotic resistance of *Mycobacterium tuberculosis* (*Mtb*) poses the need of deeper understanding of the resistance mechanism of the used therapeutics and of proposing alternatives for treatment. Previously we identified hydroxyanthraquinones (pseudopurpurin, munjistin, purpurin, xanthopurpurin) from *Rubia* spp. as structurally similar to synthetic antibacterial agents from the group of fluoroquinolones (gatifloxacin, levofloxacin, moxifloxacin). Thus, *Mtb* DNA gyrase was suggested as a new pharmacological target of hydroxyanthraquinones [1]. In this work we report further *in silico* and *in vitro* studies of fluoroquinolone antibiotics and hydroxyanthraquinones. We performed molecular dynamics simulation of the wild-type *Mtb* DNA-gyrase complexes and A90S fluoroquinolone-sensitized mutants with moxifloxacin (Mox), gatifloxacin (Gat), and levofloxacin (Lev). Mox and Gat showed stronger interactions with the enzyme in agreement with clinically recorded drug effects. As expected, in all complexes, Mg⁺⁺ had the highest contribution to the binding energies. To validate the *in silico* results, methanol extracts of *Rubia cordifolia* roots were tested on the *Mtb* strain H37Ra - ATCC[®] 25177 (MIC = 78 µg/mL and MBC = 312 µg/mL), proving the potential of the tested anthraquinone fractions to inhibit *Mtb*. Preliminary screening of the chemical composition of these fractions showed presence of anthraquinones typical for the species.

Our results help to explain the experimental observations and outline *Rubia* hydroanthraquinones as promising lead compounds with potential for anti-*Mtb* activity.

In addition to this study, the coordinating activities of the *in-silico* groups in the COST Action CA21145 (EURESTOP) will be presented.

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Synthesis of Phthalazine Derivatives: Biological Evaluation and Molecular Insights for Alzheimer's Disease

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Alzheimer's disease (AD) remains a profound challenge in neurodegenerative disorders, with its global prevalence set to rise significantly. Characterized by memory loss, cognitive decline, and behavioral changes, AD primarily affects the elderly, although early onset can occur [1,2]. The pathophysiology of AD involves beta-amyloid plaques, tau tangles, neuroinflammation, and synaptic dysfunction, contributing to progressive neuronal damage. Despite numerous therapeutic attempts, effective treatment options are limited, underscoring the urgent need for novel interventions [3]. Phthalazine-1,4-dione derivatives have emerged as promising candidates due to their ability to inhibit acetylcholinesterase (AChE), a key enzyme implicated in AD. This study focuses on the synthesis, characterization, and evaluation of new phthalazine-1,4-dione derivatives as potential AChE inhibitors [4]. Thirty-two compounds were synthesized, and extensive biological assays revealed that thirteen showed over 50% inhibition of AChE without affecting butyrylcholinesterase (BChE). Compounds 8m, 8n, and 8p demonstrated significant inhibitory activity and favorable binding affinity to AChE, comparable to donepezil, a standard AD treatment [5]. Molecular dynamics simulations confirmed the stability of these complexes, while DFT analysis highlighted their chemical stability. These findings suggest that phthalazine-1,4-dione derivatives hold potential as effective AD therapeutics, warranting further research to optimize their pharmacokinetic profiles and validate their clinical efficacy.

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