

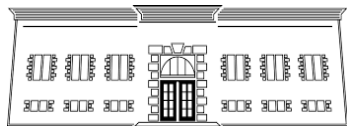
UNIVERSITETI I MJEKËSISË, TIRANË



## AGENDA Tirane, 17 June

### CA21145 - European Network for diagnosis and treatment of antibiotic-resistant bacterial infections (EURESTOP)

- 08:15 – 09:00 Registration
- 09:00 – 09:30 **Welcome – Prof. Xheladin Draçini**, Rector of University of Medicine, Tirane  
**Prof. Margarita Gjata**, Dean of Faculty of Medicine  
**Prof. Dr Suela Kelliçi**, Vice-Rector and Head of Pharmacy Department ,  
**Mrs. Mirela Muça**. Director of the National Albanian Research Agency ,  
**Mrs. Xhiliola Biçeku**, Director of Quality Assurance Agency in Higher Education, Albania,  
**Prof. Dr Mattia Mori**.EURESTOP Chair
- 09:30 – 10:00 **Oltiana Petri**, Cambridge Clinical Laboratories, Albania – **Multidrug-resistant bacteria in clinical samples**
- 10:00 – 10:30 **Esra Tatar**, Istanbul Kent University, Turkey – **Evaluation of the efficacy of silver nanoparticle and anti-MRSA effective N-(Arylsulfonyl)-L-methionine derivative acylhydrazone-loaded nanofibers for wound healing**
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13:00 – 14:15 *Lunch at your own*

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**Blue underlined speakers are YR&Is, competing for the best oral presentation award (evaluated by Core Group members attending the meeting).**



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# ABSTRACT BOOK

CA21145 - European Network for diagnosis and treatment of antibiotic-resistant bacterial infections (EURESTOP)



17 June 2025, Tirana, Albania

Hosted by University of Medicine Tirane



UNIVERSITETI I MJKËSISË, TIRANË



## 3<sup>rd</sup> EURESTOP WG MEETING

(Grant Period 3)

17 June 2025, Tirana, Albania

### Scientific Committee:

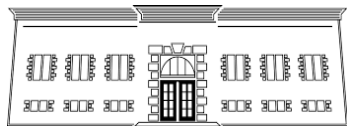
Mattia Mori (Italy) - Action Chair  
Patricia Rijo (Portugal) - Vice Chair  
Cristina Nativi (Italy) - Science Communication coordinator  
Priyanka Sahariah (Iceland) - Grant Awarding coordinator  
Dana Reichmann (Israel) - WG1 Leader  
Rossella Grande (Italy) – WG1 co-Leader  
Martijn Riool (Germany) – WG1 co-Leader  
Younes Smani (Spain) - WG2 Leader  
Carole Devaux (Luxembourg) - WG3 Leader  
Tomislav Mestrovic (Croatia) – Stakeholders Coordinator  
Didem Sen Karaman (Turkey) – YR&Is Coordinator  
Stephen Hawser (Switzerland) – Science Strategy Advisor

### Organizing Committee:

Entela Haloci (AL MC member)  
Suela Kellici (Vice rector of UMT)  
Stela Panteqi (AI MC member)  
Vilma Papajani (UMT)  
Mirela Miraçi(UMT)  
Ledjan Malaj (UMT)  
Ela Hoti(UMT)  
Bruna Myftari(UMT)  
Iris Hoxha(UMT)  
Erjon Troja (UMT)  
Linda Mato (UMT)  
Klejda Harasani(UMT)  
Nineta Saraci (WG member)  
Adela Alushi (invited)  
Meri Vasha (UMT)  
Klajdi Kellici (UMT)  
Anila Prendi (UMT)

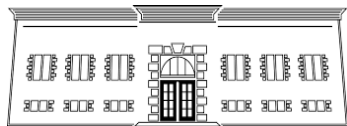


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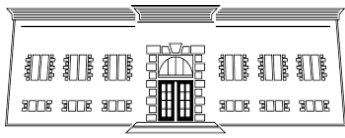
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## **ABSTRACTS (in order of presentation)**



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## MULTIDRUG-RESISTANT BACTERIA IN CLINICAL SAMPLES

**Prof.Asc. Oltiana Petri<sup>a,b</sup>, Dr. Arjana Marku<sup>a</sup>, Dr. Esmeralda Angjeli<sup>a</sup>**

a-Laboratory of Microbiology, Cambridge Clinical Laboratories, Tirana, Albania

b-Faculty of Rehabilitation Science, Sports University of Tirana, Albania

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According to WHO, antimicrobial resistance (AMR) is one of the top 10 global public health threats facing humanity. Multidrug-resistant bacteria (MDR) globally challenge clinicians and nosocomial infection control personnel due to limited treatment options and the need to implement barrier precautions to prevent their transmission. Nosocomial transmission is a major reason why the incidence of MDR is increasing. Cambridge Clinical Laboratories, from January 2024 to April 2025, we analysed 23,305 different samples from ages 0-90, such as blood cultures, wound cultures, urine cultures, cultures from various catheters, and outpatient samples. Identification and antimicrobial susceptibility testing were performed with Vitek 2 Compact and disk diffusion according to EUCAST.

Our data showed that out of 23305 samples tested, 165 were resistant to at least 3 different classes of antimicrobials. Of these, 118 were hospital samples, while 47 were outpatient. The highest percentage of Multidrug-resistant gram-negative bacteria (MRGN) cases was in males compared to females, 58% vs 42%. Of the isolated strains, MRGN lead with E. coli 36 strains, followed by K. pneumoniae and P. aeruginosa with 31 and 29 strains respectively; while S. aureus MRSA dominates in Gram positive bacteria with 43 strains. It should be considered that 6 strains are colistin resistant and 2 Enterococcus spp. strains are VRE. This study showed that the prevalence of MRGN strains is higher than the occurrence of MRSA and VRE and that the structured implementation of MRGN screening in patients at risk, strict basic hygiene, targeted isolation and adequate calculated antibiotic therapy are essential measures in managing the problem of MRGN and MRSA in outpatients and inpatients.

### References

- 1- [https://www.eucast.org/mic\\_and\\_zone\\_distributions\\_and\\_ecoffs](https://www.eucast.org/mic_and_zone_distributions_and_ecoffs)
- 2- <https://pmc.ncbi.nlm.nih.gov/articles/PMC9012092/>
- 3- <https://www.infektionsschutz.de/en/erregersteckbriefe/mrgn/>
- 4- [https://www.researchgate.net/publication/359977512\\_High\\_prevalence\\_of\\_multidrug-resistant\\_Gram-negative\\_bacteria\\_carriage\\_in\\_children\\_screened\\_prospectively\\_for\\_multidrug\\_resistant\\_organisms\\_at\\_admission\\_to\\_a\\_paediatric\\_hospital\\_Hamburg\\_Germany\\_Septem](https://www.researchgate.net/publication/359977512_High_prevalence_of_multidrug-resistant_Gram-negative_bacteria_carriage_in_children_screened_prospectively_for_multidrug_resistant_organisms_at_admission_to_a_paediatric_hospital_Hamburg_Germany_Septem)
- 5- <https://www.who.int/>



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## Evaluation of the efficacy of silver nanoparticle and anti-MRSA effective N-(Arylsulfonyl)-L-methionine derivative acylhydrazone-loaded nanofibers for wound healing

Aslıhan Yılmaz<sup>a</sup>, Bilge Tuzcu<sup>b</sup>, Gülsüm Ercan<sup>b</sup>, Hümeyra Betül Yekeler<sup>c</sup>, Ece Güler<sup>b</sup>, Erkan Rayaman<sup>d</sup>, Muhammet Emin Çam<sup>b</sup>, **Esra Tatar<sup>b</sup>**

<sup>a</sup>Department of Pharmaceutical Chemistry, Institute of Health Sciences, Marmara University, Istanbul, Türkiye

<sup>b</sup> Faculty of Pharmacy, Istanbul Kent University, Istanbul, 34406, Türkiye

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious threat to human life due to its high contagiousness and treatment difficulty, and MRSA is detected in 40% of samples taken from chronic wounds. MRSA, which is rapidly developing resistance to all known antibiotics, has also developed resistance to vancomycin and this fact has increased the danger to the highest level. Therefore, it is necessary to develop new highly effective and non-toxic drug active substances for the treatment of MRSA-infected wounds (1). In a previous work, we reported the anti-MRSA MIC value of N-[(2S)-1-[2-[(5-nitrofuran-2-yl)methylidene]hydrazinyl]-4-(methylsulfonyl)-1-oxobutan-2-yl]-4-methylbenzenesulfonamide as 3.9 µg/mL (2). We will synthesize a new series by keeping the acylhydrazone structure bearing 5-nitrofurfurylidene moiety but tosylating the amine group of L-methionine with various agents to achieve the derivative with highest anti-MRSA activity. We will prepare a wound dressing containing nanofibers in which both antibacterial silver nanoparticles (AgNP) and the most anti-MRSA active acylhydrazone derivative of our novel series will be embedded. AgNP-embedded acyl hydrazone-loaded nanofibers will be prepared by pressurized gyration (PG) method. The cytotoxic effects of AgNP and acyl hydrazone-loaded fibers will be determined by performing MTT testing. In the final stage, an in vitro wound healing test (Scratch Test) will be performed and the wound healing effectiveness of the wound dressing prepared from AgNP-embedded acyl hydrazone-loaded nanofibers will be evaluated by determining its effect on TNF-α production in L929 mouse fibroblast cells by biochemical methods.

### References

1. Int J Nanomedicine. 2023;18:4663-4679.
2. Turk J Chem. 2016; 40(13):13.



## An XDR, NDM-1-producing *Pseudomonas aeruginosa* strain of feline origin in Greece

George Valiakos<sup>a</sup>, Marios Lysitsas<sup>a</sup>, Antonis Giakountis<sup>b</sup>

<sup>a</sup>Faculty of Veterinary Medicine, University of Thessaly

<sup>b</sup>Department of Biochemistry and Biotechnology, University of Thessaly

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A backyard cat with symptoms of otitis was brought to a veterinary clinic in Central Greece. A sample was obtained, and *P. aeruginosa* was isolated. The strain exhibited an extensively drug-resistant (XDR) profile, as it was non-susceptible to all tested agents except colistin. DNA extraction and whole-genome sequencing (WGS) were performed using a robotic extractor and Ion Torrent technology, respectively. The genome was assembled and screened for resistance and virulence determinants. The isolate belonged to the ST308 clone with a total of 67 antibiotic resistance genes (ARGs) and 221 virulence factor-related genes identified. No plasmids were detected. The metallo-beta-lactamase (MBL) bla\_NDM-1 gene and 46 efflux pumps were included in the strain's resistome. Both ARGs conferring tolerance to disinfecting agents and biofilm-related genes were identified, associated with this clone's ability to adapt and persist in healthcare settings. This case demonstrates the potential for bacterial clones to spread within the community and be transmitted to companion animals, leading to opportunistic infections in susceptible individuals and further transmission to their owners, other animals, and the environment.

### References

1. Abdullahi, I.N.; Mejri, S.; Okwume, C.C.; Lawal, N.A.; Olusegun, O.A.; Sallem, R.B.; Slama, K.B. Global Epidemiology of High Priority and Pandemic *Pseudomonas Aeruginosa* in Pets, Livestock, Wild, and Aquatic Animals: A Systematic Review and Meta-Analysis. *Letters in Applied Microbiology* **2025**, *78*, ovaf028, doi:10.1093/lambio/ovaf028.
2. Hayashi, W.; Izumi, K.; Yoshida, S.; Takizawa, S.; Sakaguchi, K.; Iyori, K.; Minoshima, K.; Takano, S.; Kitagawa, M.; Nagano, Y.; et al. Antimicrobial Resistance and Type III Secretion System Virulotypes of *Pseudomonas Aeruginosa* Isolates from Dogs and Cats in Primary Veterinary Hospitals in Japan: Identification of the International High-Risk Clone Sequence Type 235. *Microbiol Spectr* **2021**, *9*, e00408-21, doi:10.1128/Spectrum.00408-21.
3. Lorusso, A.B.; Carrara, J.A.; Barroso, C.D.N.; Tuon, F.F.; Faoro, H. Role of Efflux Pumps on Antimicrobial Resistance in *Pseudomonas Aeruginosa*. *IJMS* **2022**, *23*, 15779, doi:10.3390/ijms232415779.
4. Lysitsas, M.; Triantafillou, E.; Chatzipanagiotidou, I.; Antoniou, K.; Valiakos, G. Antimicrobial Susceptibility Profiles of *Acinetobacter Baumannii* Strains, Isolated from Clinical Cases of Companion Animals in Greece. *Veterinary Sciences* **2023**, *10*, 635, doi:10.3390/vetsci10110635
5. WHO Bacterial Priority Pathogens List 2024: Bacterial Pathogens of Public Health Importance, to Guide Research, Development, and Strategies to Prevent and Control Antimicrobial Resistance; 1st ed.; World Health Organization: Geneva, **2024**, ISBN 978-92-4-009346-1.



## Investigation of the concordance between bacteremia caused by extended-spectrum beta-lactamase-producing Enterobacterales and surveillance cultures in hospitalized patients: A 5-year retrospective study

Meltem Ayaş<sup>a</sup>, Neval Yurttutan Uyar<sup>b</sup>, Sesin Kocagöz<sup>c</sup>

<sup>a</sup>Acibadem Mehmet Ali Aydınlar University, Vocational School of Health Services, Medical Laboratory Techniques Program, Istanbul, Türkiye.

<sup>b</sup>Acibadem Labmed Laboratories, Istanbul, Türkiye.

<sup>c</sup>Department of Infectious Diseases and Clinical Microbiology, Acibadem Mehmet Ali Aydınlar University, Faculty of Medicine, Istanbul, Türkiye.

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**Background:** Bloodstream infections due to extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E) have been associated with increased hospital costs, length of stay and patient mortality. ESBL-E colonization is a serious risk factor for acquiring these infections. However, the role of routine patient screening for ESBL-E colonization in predicting related infection is unclear (1,2). The aim of this study was to analyse the concordance between rectal screening isolates and ESBL-E isolates in blood culture in hospitalized patients.

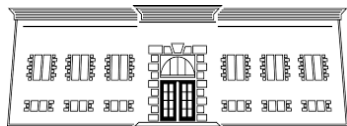
**Method:** Blood culture and rectal swab culture from 652 patients hospitalized in Acibadem Health Group hospitals between June 2019 and June 2024 were analysed. Only the first positive blood culture and the most recent rectal surveillance culture prior to the bloodstream infection were included. Rectal swabs were cultured on Chromagar ESBL, and isolates were identified using MALDI-TOF MS. Antibiotic susceptibility and ESBL production were tested according to EUCAST guidelines. Blood cultures were processed with automated systems.

**Results and conclusion:** 309 of 652 patients were positive for ESBL-producing Enterobacterales in surveillance cultures, while 343 were negative. Of the 309 patients with positive surveillance cultures, 64 (20.7%) developed bacteremia caused by the same ESBL-producing Enterobacterales in the surveillance culture. In contrast, 47 (13.7%) of 343 patients with negative surveillance cultures developed bacteremia due to non-ESBL-producing Enterobacterales. Regarding the presence or absence of ESBL-producing Enterobacterales, concordant results were obtained between surveillance and blood cultures in 111 (17%) patients.

In conclusion, bacteremia was found to be secondary to colonization. Analysis of larger cohorts with clinical outcomes is needed to identify ESBL-E rectal colonization as a major determinant of the risk of developing other infections, especially bacteremia.

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## Antibiotics availability: Drug Reimbursement List and Hospital use

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### Introduction:

In Albania, every drug that is granted a Marketing Authorization is made available to patients through three primary channels. Antibiotics, as a class of drugs, are dispensed to patients at pharmacies exclusively with a valid prescription issued by licence medical professionals. These prescriptions according to the law should be written using the international nonproprietary name (INN). Antibiotics included in the national Drug Reimbursement List may be obtained by patients either with full reimbursement or with a co-payment. A third category includes, antibiotics administered in public and private healthcare hospitals for inpatient treatment only, (hospital use).

### Purpose:

The objective of this study is to analyse the antibiotics registered in Albania that are part of the Drug Reimbursement List and those included in the approved list of medicines for hospital use. The analysis focuses on measurable consumption patterns based on reimbursement data and hospital procurement requests, covering a one-year period.

### Methodology:

The analysis identifies all antibiotics with a marketing authorization in Albania, using data from the National Drug Register (Ministry of Health and Social Protection, National Agency for Medicines and Medical Devices), the current Drug Reimbursement List (Health Insurance Fund), and the approved List of Medicines for Hospital Use at the national level.

### Results:

Although a large number of antibiotics are registered in the Albania, but some of them are not imported or available on the market. The number of antibiotics included in the Drug Reimbursement List (17 antibiotics) is relatively small in comparison to those that have a marketing authorization(), imported, and prescribed by general practitioners. In contrast, the List of Medicines for Hospital Use shows an upward trend in both the quantity and variety of antibiotics administered in hospitals, including newer generations of these drugs.

### Conclusions:

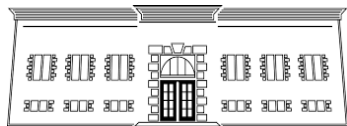
There has been a noticeable increase in applications for the registration of new antibiotics submitted to the National Agency for Medicines and Medical Devices. This trend is accompanied by a rising demand in the open pharmaceutical market, greater levels of reimbursement, and higher consumption by public healthcare institutions—particularly of newer-generation antibiotics. These developments have contributed to a rise in antibiotic resistance, especially within hospital settings.

### Keywords:

Antibiotics in Albania; Drug Reimbursement List; Hospital Medicines List; Health System; Legal Framework; Pharmaceutical Policy.

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## Design and Evaluation of Novel Dihydropyrimidine-Based Hybrids as Antibacterial Agents Targeting DNA Gyrase

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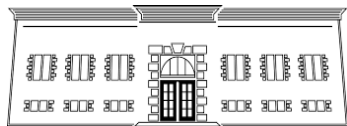
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In an effort to develop new antibacterial candidates, we synthesized a series of hybrid molecules incorporating 1,4-dihydropyrimidine scaffolds—recognized quinolone isosteres—fused with pyridine and pyrimidine moieties [1-2]. The rationale for this design stems from the well-established mechanism of quinolones, which exert antibacterial effects through DNA gyrase inhibition [3-5]. Two distinct classes of derivatives were prepared: 2-[(5-cyano-6-oxo-6-(pyridin-4-yl)-1,6-dihydropyrimidin-2-yl)-N-(benzothiazol-2-yl)-acetamides (5a–5g) and 2-[(5-cyano-6-oxo-6-(pyridin-4-yl)-1,6-dihydropyrimidin-2-yl)thio]-N-(thiazol-2-yl)acetamides (6a–6f). Their antibacterial efficacy was assessed through in vitro assays, where compounds 5a, 6b, and 6c exhibited potent inhibitory activity. Particularly, 6b and 6c displayed strong effects against *Escherichia coli*, with MIC values of 1.95 and 0.97 µg/mL, respectively—comparable to standard antibiotics. Compound 5a also demonstrated significant action against *Enterococcus faecalis*. Gel-based DNA gyrase inhibition assays further confirmed that these compounds effectively prevent supercoiled DNA formation, indicating a mechanism aligned with DNA gyrase inhibition. Molecular docking and dynamics simulations supported these findings, highlighting key interactions with the gyrase active site. Overall, these novel hybrids hold promise for further development as antibacterial agents, potentially expanding the therapeutic options against resistant bacterial strains.

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## Azo derivatives of monoterpenes as anti-*Helicobacter pylori* agents: synthesis, characterization, antimicrobial properties and structure-based target investigation

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*Helicobacter pylori* (Hp) infection is a global health concern. Current therapeutic arsenal includes antibiotics and proton pump inhibitors, despite this drug combination did not limit the spread of antimicrobial resistance. Natural phenolic monoterpenes (eugenol, vanillin, carvacrol, and thymol) displayed antimicrobial properties, low cost and chemical versatility. Thus, we aimed at the functionalization of these small compounds through diazotization reaction, achieving a large series of mono- and bis-azo derivatives. The compounds were tested against four Hp strains including three clinical isolates, providing some potent and selective inhibitors. Thus, the most promising compounds underwent *in vitro* cytotoxicity evaluation on two normal cell lines and putative target investigation by carrying out a structure-based approach based on docking calculations on four targets for Hp, namely urease,  $\beta$ -hydroxyacyl-acyl carrier protein dehydratase, glucose 6-phosphate dehydrogenase, and inosine 5'-monophosphate dehydrogenase. Lastly, some azo derivatives were also studied for their photoisomerization process and liposome encapsulation to shed light on their chemical-physical properties and the impact on liposomal membrane permeability for further applications in photodynamic therapy.

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## Effects of Polyphenol and Antibiotic Combinations on MRSA Standard and Clinical Strains: Preliminary Findings

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Pathogenic microorganisms—especially antibiotic-resistant strains such as Methicillin-Resistant *Staphylococcus aureus* (MRSA)—cause infections that are increasingly difficult to treat with conventional antibiotics. Therefore, new strategies are needed to combat such infections. One of these strategies involves using different antimicrobial agents in combination.

In this study, the phenolic compound gallic acid was combined with various antibiotics such as cefazolin, kanamycin, and ampicillin. Fifteen different MRSA clinical isolates and a standard strain were evaluated using various tests.

The antibiotic susceptibility profiles of the MRSA strains were analyzed using the Kirby-Bauer disk diffusion method. MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values were determined for the standard strain. To evaluate the effectiveness of the combinations, combined well diffusion and checkerboard tests were applied.

The combination of gallic acid with antibiotics—particularly tetracycline, ampicillin, kanamycin, and chloramphenicol—significantly increased antimicrobial activity compared to the antibiotics used alone. In the checkerboard test, kanamycin and chloramphenicol showed an additive effect with gallic acid, with FICI (Fractional Inhibitory Concentration Index) values ranging between 0.5 and 1.

These preliminary findings suggest that polyphenolic compounds used in combination with antibiotics may offer a promising therapeutic approach in the fight against MRSA infections.

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## Targeting BambL and LecA with multivalent glycomimetics: assessing complex stability and lectin bridging using computational tools

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Polymicrobial infections, such as those frequently observed in cystic fibrosis patients, generally involve coexisting pathogens. For example, *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex species cooperate to enhance biofilm formation and exacerbate host inflammatory responses.<sup>1</sup> These bacteria rely on carbohydrate-binding lectins—such as LecA (galactose-binding) and BambL (fucose-binding)—to mediate adhesion, colonization, and biofilm development on host tissues. LecA is directly implicated in epithelial adhesion, cytotoxicity, and biofilm maturation<sup>2</sup>, while BambL is a highly multivalent lectin involved in fucosylated glycan recognition, with structural features supporting its role in host colonization<sup>3</sup>. Disrupting these lectin-glycan interactions represents a promising non-antibiotic strategy to dampen bacterial virulence.<sup>4</sup> In this study, we employed molecular docking and long-timescale molecular dynamics simulations to explore the capacity of rationally designed trivalent ligands to engage both BambL and LecA simultaneously. The ligands, featuring two fucosyl and one galactosyl moieties respectively, were shown to stably interact with distinct binding pockets within the same BambL trimer and simultaneously engage both BambL and LecA, supporting their potential to mediate dual lectin binding across different protein targets. Notably, the simulations support the structural feasibility of a bridging effect between BambL and LecA.

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## Steroidal hormones and neurosteroids as adjuvant-like compounds in bacterial infections

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Steroid hormones and neurosteroids are lipophilic compounds that modulate a variety of physiological processes in humans, including immune function and microbial susceptibility. Emerging evidence suggests that certain steroidal structures may also interact with bacterial efflux pumps, key mediators of antibiotic resistance. In this study, we evaluated a structurally diverse library of endogenous and synthetic steroidal compounds, focusing on their potential to inhibit efflux-mediated resistance in bacterial pathogens. Structure–activity relationship (SAR) analysis identified pregnanolone as a lead compound, and further modifications at C-3 and C-17 positions yielded enhanced derivatives, including UCT-032 and UCT-069. These compounds demonstrated the ability to overcome resistance mechanisms *in vitro*, likely through interference with bacterial efflux systems. Notably, VŠCHT-069 displayed additive effects with antibiotics against clinical isolates of resistant strains, without apparent cytotoxicity.

Transcriptomic analysis of *Staphylococcus aureus* exposed to UCT-069, erythromycin, ciprofloxacin, and their combinations revealed that the steroid alone downregulated genes related to virulence. In combination with erythromycin, UCT-069 significantly suppressed ribosome-related gene expression, supporting a mechanism of increased antibiotic sensitivity through suppression of protein biosynthesis. However, no clear effect on efflux pump gene expression was observed. These findings highlight the potential of neurosteroid-based compounds as antibiotic adjuvants that may restore antibiotic efficacy by modulating bacterial gene expression and resistance phenotypes.

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## Abietane derivatives: A New Approach to Resistant Skin Infections

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Antimicrobial resistance poses a major health threat, as the effectiveness of existing antibiotics continues to decline, leading to more frequent treatment failures. Two abietane diterpenoids, 7 $\alpha$ -acetoxy-6 $\beta$ -hydroxyroyleanone (**Roy**) and 6,7-dehydroroyleanone (**DeRoy**), isolated from *Plectranthus* spp. have demonstrated notable antibacterial activity, especially against methicillin-resistant *Staphylococcus aureus* (MRSA). In addition, three **DeRoy**-based compounds have also demonstrated promising antimicrobial properties along with low cytotoxicity. These findings suggest that these compounds could be promising options for fighting antibiotic-resistant bacteria. Building on these observations, this study explores the use of royleanone derivatives to target pathogenic bacteria linked to skin problems, while also protecting the skin's beneficial microbes to help keep its natural balance. An in-house library of approximately 40 royleanone derivatives was developed based on the lead compounds **Roy** and **DeRoy**. These compounds are currently being evaluated through antimicrobial assays, including minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and biofilm inhibition tests. Preliminary screening has identified four derivatives with strong antibacterial activity against MRSA and low cytotoxic, highlighting their potential for further development. Investigation into the mechanism of action suggest that **Roy** primarily targets the bacterial cell wall rather than the cell membrane. Although **Roy** is capable of interacting with phospholipid membranes, it does not appear to cause significant membrane disruption. This indicates that its antibacterial activity may involve intracellular pathways or inhibition of cell wall synthesis, rather than compromising membrane integrity. This study supports the development of new skin treatments that target harmful bacteria like MRSA while preserving the skin's natural microbiota. Royleanone derivatives show strong potential as safe and effective alternatives for microbiota-related skin conditions.

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## Identification of potential dual drug efflux pump inhibitors in bacteria and resistant cancer cells: a protocol for systematic review

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Drug extrusion mediated by membrane transporters acting as efflux pumps is one of the major mechanisms conferring multidrug resistance (MDR) both in bacteria and in cancer cells [1]. The identification of dual efflux pump inhibitors (EPIs) that reverse resistance in bacteria and in tumor cells is an intriguing research topic, however no systematic exploration of compounds with EPI activity from either independent or simultaneous experiments in bacteria and cancer cells has been reported so far. Therefore, we aim to conduct a systematic review of recent scientific literature containing data for inhibitors targeting bacterial and mammalian efflux pumps. For this purpose, we developed a protocol for a systematic literature review in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines [2]. Pilot searches in the literature (PubMed and Scopus databases) and abstract screening resulted in identification of 763 unique records. Of these, 246 records were selected for the next steps of full-text review and data aggregation on structural and biochemical properties of the compounds.

We expect that our review will highlight the overlap between EPIs in bacterial and cancer cell as a strategy to combat MDR in both settings. Further, it will provide structured data to facilitate rational design of dual (bacterial and cancer) EPIs through *in silico* ligand- and structure-based approaches. Additionally, the outcomes of this review may support the concept of using bacteria as a model to screen and evaluate EPIs that could potentially be used to overcome MDR in cancer cells. Finally, the results will generate perspectives for future research efforts in tackling MDR with potential clinical implications.

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## Physicochemical, toxicological and antifungal properties of some 6-chloro-1,3,5-triazine-2,4-diamine derivatives with alkyl and cycloalkyl substituents

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Triazine derivatives are well-known biologically active compounds, widely used in medicine and agriculture due to their antibacterial, antifungal, antiviral, herbicide and anticancer activity [1]. As a fundamental heterocyclic moiety, the triazine ring holds a significant pharmacological relevance in drug design. Symmetrical triazine (*s*-triazine or 1,3,5-triazine) serves as an excellent foundation for development of novel biologically active compounds. The present research focuses on the *s*-triazine structures given in Fig. 1, which include 6-chloro-1,3,5-triazine-2,4-diamine derivatives with alkyl and cycloalkyl substituents. These compounds underwent physicochemical characterization and antifungal analysis [2-5] demonstrating promising properties for further advanced investigations. A preliminary study [2] revealed their significant antifungal potential towards *Aspergillus flavus* with molecular docking analysis predicting a possible mechanism of action. The compounds with cycloalkyl substituents exhibited slightly weaker antifungal activity than compounds with alkyl substituents, but lower toxicity. Further comprehensive evaluation of their biological potential is essential for determining their possible practical applications.

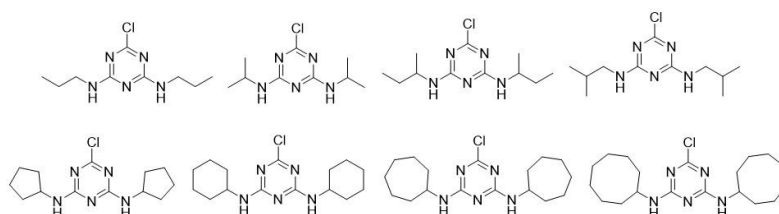
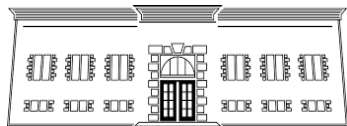


Figure 1. Molecular structures of the analyzed triazine derivatives

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## Functional high-throughput screening identifies microRNAs regulating infection by *Staphylococcus aureus*

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Infection by the opportunistic bacterial pathogen *Staphylococcus aureus* can lead to various outcomes, from mild skin diseases to severe invasive conditions. The emergence and dissemination of antibiotic-resistant strains in clinical and community environments have positioned *S. aureus* infections as the leading cause of death among bacterial infectious agents worldwide<sup>1</sup>. A better understanding of the complex host-pathogen relationship is crucial to propose alternative antibacterial treatments against virulent and drug-resistant *S. aureus* isolates, such as methicillin-resistant *S. aureus* (MRSA) strains. As a facultative intracellular pathogen, *S. aureus* can resist drug treatment and escape the immune response by invading, replicating and persisting within a variety of human cells<sup>2</sup>. Notwithstanding, the host factors sustaining *S. aureus* intracellular lifestyle are largely unknown.

MicroRNAs are a well-studied class of host genome-encoded small non-coding RNAs that play a pervasive role in the post-transcriptional control of eukaryotic gene expression. MicroRNAs have emerged as important players in the interplay between bacterial pathogens and host cells<sup>3</sup>, although a comprehensive analysis of the impact of microRNAs on infection by *S. aureus* is still missing.

To identify microRNAs that modulate *S. aureus* infection of epithelial cells, we performed a fluorescence microscopy-based functional screening using a genome-wide library of miRNA mimics (2,042 human mature microRNAs). We selected 87 miRNAs that strongly determine the outcome of the infection by *S. aureus*, with pro-infective or anti-infective profiles. The effect of the top-scoring microRNAs modulating *S. aureus* infection was validated in infection with three clinical isolates, which were collected from patients with different clinical manifestations and present distinct intracellular lifestyles<sup>4</sup>. In addition, time-course experiments demonstrated that distinct stages of *S. aureus* infection cycle, namely invasion, vacuolar escape and replication, are affected by specific microRNAs. Interestingly, a comparison of the screening results for *S. aureus* with those previously performed in the laboratory for two other bacterial pathogens - *Salmonella* Typhimurium and *Shigella flexneri*, revealed that non-overlapping subsets of microRNAs control infection by these pathogens.

Overall, our work demonstrates the key role played by host microRNAs in orchestrating host cell responses to bacterial infections, as well as the value of using functional high-throughput screenings to identify novel molecular players governing the complex interaction between host and bacterial pathogens and, ultimately, pathogenesis.

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## Oral microbiota assessment in adolescent patients with MALDI-TOF technology

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The oral microbiota has an important role in maintaining oral and systemic health. Its composition can be significantly altered during orthodontic treatment in adolescents. This study evaluates the oral microbiota in adolescent patients using MALDI-TOF VITEK technology, a mass spectrometry-based method for microbial identification.

Saliva and plaque samples were analyzed in our university patients, with and without fixed orthodontic appliances from 12-18 years old. We identified shifts in microbial diversity and abundance which were confirmed with the MALDI TOF technology. After identifying the oral pathogens in the same moment we also do the antibiogram to choose the best empiric antimicrobial treatment. The findings highlight correlations between orthodontic treatment and the prevalence of pathogenic microorganisms, offering insights into potential risks such as caries and periodontal diseases. Changes in oral microbiota during orthodontic treatment can have several implications for oral health: Increased risk of dental caries, gingival inflammation and periodontal disease, White Spot Lesions (WSLs), halitosis, systemic health implications, respiratory infections etc. Maintaining a balanced oral microbiota before and during orthodontic treatment involves adopting healthy habits and practices that support both oral and overall well-being. Practicing good oral hygiene at home and professional dental cleaning once in two months helps reduce the microbiota shifts after bonding orthodontic appliances.

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## Structural and Biological Evaluation of Chitosan and Crosslinked Chitosan Coated Fe<sub>3</sub>O<sub>4</sub> Nanoparticles for Anticancer Drug Delivery

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Nanosized magnetite nanoparticles (Fe<sub>3</sub>O<sub>4</sub> NPs) are widely utilized in cancer research as drug carriers due to their low toxicity, stability in aqueous media, biocompatibility, biodegradability, and superparamagnetic properties [1, 2]. However, unmodified NPs often exhibit high toxicity and poor colloidal stability in physiological environments [3]. To maintain particle size over time and prevent aggregation, the surface of Fe<sub>3</sub>O<sub>4</sub> NPs is typically coated with polymers [5]. In this study, we focused on the synthesis and characterization of Fe<sub>3</sub>O<sub>4</sub> NPs surface-coated with chitosan and its crosslinked derivative. These NPs were subsequently loaded with the natural anticancer drug, 5,7-dihydroxyflavone to enhance their therapeutic efficacy. X-ray diffraction (XRD), ultraviolet-visible (UV-Vis), and in vitro analyses were conducted to thoroughly evaluate the structural, physicochemical, and biological properties of the samples. XRD analysis confirmed the crystalline nature of the magnetite core through characteristic peaks, with the average crystallite size measured at approximately 13 nm for chitosan-coated Fe<sub>3</sub>O<sub>4</sub> NPs (Fe<sub>3</sub>O<sub>4</sub>-CS) and 17 nm for crosslinked chitosan-coated Fe<sub>3</sub>O<sub>4</sub> NPs (Fe<sub>3</sub>O<sub>4</sub>-CL-CS), both within the optimal size range for biomedical applications. UV-Vis spectroscopy demonstrated a significant increase in drug-loading efficiency from 52% in Fe<sub>3</sub>O<sub>4</sub>-CS to 83% in Fe<sub>3</sub>O<sub>4</sub>-CL-CS, indicating that crosslinking induced structural changes in the polymer network that enhanced drug binding affinity and interaction. Furthermore, in vitro cytotoxicity assays using HCT 116 colorectal cancer cell lines showed that treatment with Fe<sub>3</sub>O<sub>4</sub>-CL-CS loaded with the drug resulted in significantly lower cell viability compared to treatment with Fe<sub>3</sub>O<sub>4</sub>-CS-drug samples, suggesting enhanced therapeutic potential of the crosslinked formulation. These findings highlight the advantages of polymer crosslinking in optimizing nanoparticle-based drug delivery systems for improved anticancer efficacy.

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## First report for fully synthetically fabricated surface-molecularly imprinted polymers for whole bacteria detection

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Antibiotic-resistant bacteria pose a major challenge in health care and aquaculture environments. Developing advanced diagnostic and monitoring tools is essential to address this threat. Among these, biosensors using molecularly imprinted polymers (MIPs) for direct bacterial detection stand out for their robustness, low cost, and long shelf-life. However, traditional MIP fabrication relies on fresh template bacteria and suffers from low, non-tunable imprint density and distribution [1].

In this work, thanks to our novel “template bacteria-free” approach, we designed surface-MIPs-based biosensors with exceptionally high-density biomimetic imprints ( $3 \times 10^7$  cavities/cm<sup>2</sup>) without requiring real template bacteria. Using direct laser writing lithography, we fabricated a master mold with cavities shaped to mimic *Escherichia coli* (*E. coli*) as a model organism. Soft lithography then produced a PDMS-based positive stamp with *E. coli*-like protrusions that are then covalently coated with *E. coli*-specific lipopolysaccharides to enhance selective chemical recognition. Surface-MIP layers were formed by imprinting this stamp onto a pre-polymerized polymer matrix (e.g., polyurethane) spin-coated on various transducers.

We demonstrated the versatility and performance of this approach using three transducer types: interdigitated electrodes, quartz crystal microbalance chips, and diffraction gratings. The sensors achieved an imprinting factor of 6.5 within 15 minutes of *E. coli* exposure. Detection was validated via electrochemical impedance spectroscopy, quartz crystal microbalance with dissipation monitoring, and reflective diffraction gratings-based signal measurements.

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## Built to Break: LED-NMR Insights into Photodegradable Antibiotics

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If we could design an antibiotic with an inherent self-destruct mechanism that activates upon excretion, it would represent a significant advancement in combating antimicrobial resistance. Our research focuses on developing such antimicrobials that could photochemically degrade<sup>1–3</sup> after use, whether through organized wastewater treatment (human applications) or exposure to sunlight (veterinary applications). To ensure these compounds degrade rapidly, effectively, and safely, it is crucial to understand their photodegradation mechanisms and to identify the resulting breakdown products, thereby avoiding the formation of secondary pollutants. LED-NMR emerged as a method of choice, providing detailed information on both aspects. Oxazolidine-based compounds,<sup>4</sup> known for their antibacterial properties and susceptibility to rapid decomposition, were selected as model systems for this study. The compounds were irradiated at 310 nm, and their decomposition was monitored using LED-NMR, HRMS, and MS/MS, yielding mechanistic insights into their photochemical decomposition and illuminating their degradation pathways.<sup>5</sup>

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## Fighting Antimicrobial Resistance with Light: Exploring Photodynamic Inactivation Strategies

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Antimicrobial resistance is a global health challenge requiring innovative solutions<sup>1</sup>. Photodynamic therapy (PDT) employs photosensitizers activated by visible light in the presence of dioxygen to generate reactive oxygen species (ROS). These cytotoxic ROS interact with biomolecules in biological structures, causing cell death. PDT is already used clinically to treat tumors and has recently been applied to inactivate microorganisms, viruses, and parasites, including those responsible for neglected tropical diseases such as leishmaniasis<sup>2</sup>. Due to its multitarget, non-selective mechanism, aPDT makes the development of resistance by microorganisms highly improbable. Additionally, it effectively inactivates both antibiotic-sensitive and resistant strains<sup>3</sup>. This work highlights significant achievements by our group in aPDT, emphasizing porphyrin-based photosensitizers and key biological results against resistant and sensitive bacterial strains.

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## Application of Photodynamic Inactivation against resistant plant pathogens in agriculture

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Bacteria, fungi, and pest insects present significant challenges to growers, as they cause plant diseases or spoil of crops, thereby reducing yields. The use of pesticides, including antibiotics, in agriculture has a detrimental effect on the environment and fires the development of resistance. Photodynamic Inactivation (PDI) is a powerful tool to kill microorganisms even if resistant to conventional treatment. This presentation aims at providing an overview on the application of PDI in agriculture. Formulations of sodium magnesium chlorophyllin (Chl, food additive E140) are utilised as cost-effective and environmentally sustainable photoactive agents, and illumination is performed with either LED light (395 nm) or sunlight in the range of 26.6 to 300 J/cm<sup>2</sup>. One hundred micromolar Chl induces a clear photoantimicrobial effect against the streptomycin-resistant bacterial phytopathogen *Erwinia amylovora* [2], and the fungus *Botrytis cinerea* is effectively controlled by PDI based on a formulation containing 224 µM Chl [3], even if resistant against conventional treatment. To fight the insect pest *Drosophila suzukii* Chl can be provided as food in 3% sucrose or sprayed onto insects. After feeding on 5 mM Chl (drug to light interval 9 h) and illumination with 78.9 J/cm<sup>2</sup> (LED 395 nm) 98.4% of all spotted wing *Drosophila* can be killed. Increased radiant exposure (LED 395 nm, 315.6 J/cm<sup>2</sup>) allows for usage of lower Chl concentrations (1 mM, 97.5% kill). If using sunlight (294.5 J/cm<sup>2</sup>) for Chl (5 mM) activation all flies are erased two days after illumination. Fluorescence microscopy confirms Chl accumulation in the flies' intestines [4]. Formulations of Chl can also be employed for postharvest photodecontamination of fruits, as proven by using an orange peel model and *Penicillium digitatum* as fungal model organism [5]. In conclusion, PDI based on formulations of Chl is applicable in agriculture to combat resistant bacterial and fungal plant diseases and pest insects.

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## From Plants to Pills: Formulating a Phytochemical Solution to Antibiotic-Resistant *Helicobacter pylori*

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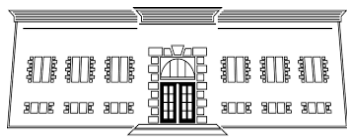
Antibiotic therapies with proton pump inhibitors, commonly used to treat *Helicobacter pylori* infection, have drawbacks such as limited efficacy, resistance development, side effects, and frequent recurrence. Due to these challenges, the WHO listed *H. pylori* as a priority for new antibiotic solutions. Essential oils (EOs) from aromatic plants offer strong antimicrobial properties and a complex composition that limits resistance development<sup>1</sup>.

This study started with screening 20 Mediterranean plant EOs for anti-*H. pylori* activity using a colorimetric microdilution assay. The most active oils—*Satureja hortensis* and *Origanum vulgare* subsp. *hirtum* (2:1 ratio)—had a MIC of 0.5  $\mu\text{L}/\text{mL}$ <sup>1</sup>. This blend eradicated *H. pylori* in 70% of infected Balb/c mice at 41 mg/kg without toxicity or immune side effects. To enhance efficacy, *Thymus vulgaris* EO was added, creating the final blend, named HerbELICO<sup>®</sup>, dominated by carvacrol (47.44%),  $\gamma$ -terpinene (18.20%), p-cymene (13.35%), and thymol (11.62%)<sup>2</sup>.

HerbELICO<sup>®</sup> was tested against 20 global *H. pylori* strains with varying antibiotic resistance and showed no resistance. A 10-minute exposure in time-kill assays inactivated all bacteria. It also penetrated a 2.5% mucin barrier, killing all *H. pylori* underneath within one hour. After encapsulation as a dietary supplement, a small case study of 15 users with *H. pylori*-positive stool tests showed a 93% eradication rate with no adverse effects<sup>3</sup>. Furthermore, a longitudinal single-patient case study was conducted on an individual with a history of antibiotic-resistant *H. pylori* infection. The patient underwent a 45-day treatment with HerbELICO<sup>®</sup>, during which quantitative urea breath tests were performed at regular intervals. A consistent decline in *H. pylori* urease activity was observed throughout the treatment period, indicating a progressive reduction of bacterial load and demonstrating the formulation's potential to eradicate even resistant strains. These results support further clinical trials of HerbELICO<sup>®</sup> as a potential routine treatment for *H. pylori* infection.

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## Dip Coating of K-Wires with Sol-Gel Based Quaternary Ammonium Integrated Antibacterial Coatings

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Kirschner wires (K-Wires) are widely used short-term implants for fracture fixation and bone stabilization in orthopedic surgery (Kim et al., 2020). However, their penetration through the skin barrier and direct contact with bone can lead to bacterial colonization, increasing the risk of infection, particularly by *Staphylococcus aureus* (Abul et al., 2023). In this context, antibacterial coatings have emerged as a critical solution for preventing infections associated with K-Wires (Oliveira et al., 2018). Various coating technologies, including plasma spraying, biomimetic deposition, electrochemical deposition, electrophoretic deposition, and sol-gel coating, are used to enhance implant surfaces' biocompatibility and antibacterial properties (Tranquillo & Bollino, 2020). In this study, the sol-gel dip coating method was employed, demonstrating its ability to provide homogeneous and defect-free coatings, thus enhancing the long-term stability of implants. The coating matrix consisted of polyvinyl alcohol (PVA), tetraethyl orthosilicate (TEOS), and quaternary ammonium silane (QAS). TEOS forms a silica network, improving mechanical stability, while QAS provides direct bacterial inhibition through high positive charge density ( $\text{NH}_4/\text{cm}^2$ ) (Kügler et al., 2005). The dip coating method ensures precise control over coating thickness and strong adhesion to the implant surface, supporting the long-term biostability of K-Wires. Experimental results demonstrated that the applied coating significantly reduced bacterial adhesion and exhibited a 55% bactericidal effect against *S. aureus*. Surface charge density measurements confirmed that QAS-modified coatings achieved sufficient electrostatic potential to disrupt bacterial cell membranes upon contact. The bacterial adhesion tests further indicated a substantial decrease in colonization compared to non-coated controls, verifying the ability of the coatings to hinder early-stage biofilm formation (Van de Lagemaat et al., 2017).

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## Enhancing Fragment-Based Drug Discovery: Cyclodextrin Complexation of Low-Solubility Natural Fragments Using Carvacrol as a Model

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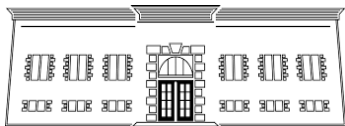
Fragment-based drug discovery (FBDD) has emerged as a robust and efficient approach in early-stage drug development. Among the diverse sources of fragment libraries, natural product-derived compounds offer unique structural features and high ligand efficiency. However, their limited aqueous solubility often hampers their utility in biophysical screening and downstream optimization.

In this study, we address this critical limitation using Carvacrol, a plant-derived phenolic compound, as a representative model. We hypothesize that cyclodextrin-based inclusion complexation can significantly enhance the aqueous solubility and overall developability of such fragments. To investigate this, we implement a comprehensive multi-scale computational framework, integrating molecular docking, extended molecular dynamics (MD) simulations, and Funnel MD techniques to elucidate the binding mechanisms and energetic landscape of the Carvacrol–cyclodextrin interaction. Insights derived from these simulations inform the design of a predictive protocol capable of quantitatively ranking cyclodextrin–fragment binding affinities, enabling the rational identification of optimal host–guest systems. Beyond improving the solubility profile of Carvacrol, this approach provides a generalizable strategy applicable to structurally related natural fragments such as Thymol, thereby supporting more informed fragment selection and optimization in FBDD pipelines.

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## Alchemical-AI for lead optimization in Drug Discovery: Lead optimization through Enhanced Alchemical Free Energy Calculations and generative AI

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G protein-coupled receptors (GPCRs) are key targets for drug development, and understanding their interactions with specialized pro-resolving mediators (SPMs) like RvD1 and RvD5n-3 DPA is essential. This talk introduces modern alchemical free energy of binding calculations, such as Free Energy Perturbation (FEP) and Thermodynamic Integration (TI) approaches, by AMBER and NAMD packages, for congeneric ligands that bind to GPCRs.

We used an AlphaFold model of the GPR32 receptor, refined it with molecular dynamics simulations, and calculated binding energies for 10 complex SPMs, achieving an average error of 2.15 kcal/mol. For RvD1, our prediction was very accurate, with an error of just 0.45 kcal/mol. We also studied transformations of RvD1 into its active form (AT-RvD1) and its less active versions, noting specific energy changes. Our investigation included RvD5n-3 DPA's binding to GPR101, revealing significant binding differences.

Despite these advancements, challenges remain in achieving consistent results with GPCR proteins. To address this, we plan to use AlphaFold3, which offers better modeling, in addition to leverage generative AI research tools to achieve robust convergence, to optimize errors and to make efficient workflows, especially for complex membrane proteins like GPCRs. This combination of alchemical free energy calculations and generative AI is expected to improve accuracy and reduce computational costs, facilitating drug discovery efforts beyond GPCR targets.

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## Galloylquinic acids disrupts biofilms and quorum sensing in multidrug-resistant *Pseudomonas aeruginosa*: From bench to pre-clinical wound healing

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### ABSTRACT

*Pseudomonas aeruginosa* is an opportunistic pathogen in wound infections, particularly in immunocompromised individuals. Its ability to form biofilms and regulate virulence through quorum sensing (QS) contributes to its multidrug resistance and persistence. This study aimed to evaluate the antimicrobial, anti-biofilm, and anti-QS potential of galloylquinic acids (GQAs), phytochemicals isolated from *Copaifera lucens*, against clinical isolates of multidrug-resistant *P. aeruginosa* from hospitalized patients *in vitro* and *in vivo*. GQAs showed strong antibacterial activity with inhibition zones of 25-40 mm, MICs of 1-4 µg/mL, and MBCs of 2-16 µg/mL. Their anti-biofilm efficacy was evident through significant inhibition of pre-formed biofilms (MBIC<sub>80</sub> = 64 µg/mL, MBEC<sub>80</sub> = 128 µg/mL), confirmed by fluorescence and confocal microscopy, showing 62.5% reduction in biofilm thickness. SEM imaging revealed structural disruption of bacterial cells and biofilm matrix. GQAs also markedly suppressed virulence traits including pyocyanin, rhamnolipids, and swarming motility. Gene expression analysis demonstrated downregulation (up to 89%) of key QS genes (*lasI*, *lasR*, *pqsA*, *pqsR*). GQAs accelerated healing of infected wounds *in vivo* and reduced bacterial burden and inflammation in rat models. These findings underscore the potential of GQAs as promising antivirulence agents for treating MDR *P. aeruginosa* infections and combating biofilm-related chronic wounds.

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## Selecting the most appropriate 3D cell culture technology to investigate bacterial-host interactions

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3D cell culture has become the *de facto* 'go-to' when determining the efficacy and toxicity, and understanding interactions between prokaryotic and eukaryotic cells.

But 3D is not just 3D. There are many 3D culture systems currently in use. Several of them have been used to investigate bacterial-host interactions. Perhaps, because of the fact that there is no single culture system that is well suited for all applications, there is a plethora of options. These include spinner cultures, hanging drop, gels (of various types), sandwich cultures, membrane divisions, low adherence microtiter plates, irrigated petri dishes, magnetic, clinostat (and other suspension) bioreactors, 'organ-on a chip' and microfluidic devices. Every system has its own advantages and disadvantages. The drawbacks represent a minefield to negotiate if one is to obtain human-bacterial relevant data. Some of these drawbacks include a wide variety of elements such as shear stress (or the lack of it), hypoxia, hyperoxia, reperfusion injury, tensile stress, nutrient starvation, metabolite toxicity, lack of complexity, environmental stiffness or lack of physical activation or an immunological element. Bacterial 'requirements', and the use of non-pathological and pathological strains also need to be taken into consideration.

3D cell culture technology is disruptive: old technologies may not be as powerful as they were with 2D cell culture, making it necessary to develop new approaches both downstream and upstream. However, coupled with the advent of pluripotent stem cells (embryonic or induced), or patient-derived (stem) cells and with CRISPR-Cas9, cell culture has gone through a paradigm shift in the last decade and can now provide an *in vitro* culture system that will respond to bacterial infections in a manner which mimics the *in vivo* response.

In this presentation, we will present a brief review of some of the factors to be considered when selecting a suitable 3D technology and give a few examples of recent successes.



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## Tracking Longitudinal Changes in Colonizing *Escherichia coli*: A Paediatric Case Study Over Nine Months

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The emergence of antimicrobial resistance (AMR) during the treatment of bacterial infections significantly reduces the chances of therapeutic success and poses a serious threat to patient health. Additionally, bacterial colonization—often a precursor to infection—not only increases the risk of subsequent infections but also serves as a reservoir for AMR genes.

In this case study, we analysed 10 *Escherichia coli* isolates collected from tracheal aspirates of a 2-year-old male patient previously diagnosed with birth asphyxia. As part of palliative care, the patient received multiple courses of prophylactic antibiotic therapy, including amoxicillin/clavulanate and cefixime.

Samples were collected over a nine-month period (09/03/2020–12/11/2020), and species identification was performed using the Vitek 2 automated system. Antibiotic susceptibility profiles were determined using both the Vitek 2 system and broth microdilution methods. All 10 *E. coli* isolates were sequenced using the Oxford Nanopore MinION platform.

Most isolates belonged to an uncharacterized sequence type closely related to *E. coli* ST131, a globally disseminated pandemic clone known for its rapid evolution and increasing antimicrobial resistance. Two isolates collected at later time points exhibited elevated resistance to several antibiotics.

All isolates were found to harbour a mega plasmid, although its size and gene content varied among samples.

These findings highlight the adaptability of colonizing bacterial populations under sustained antibiotic pressure and underscore the importance of continuous genomic surveillance in clinical settings.



## Is there a way to enhance the antimicrobial potency of the natural compound protulactone A?

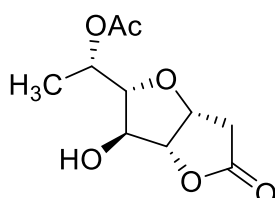
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Protulactone A (PLA), a naturally occurring fungal secondary metabolite, was isolated from EtOAc extract of the marine-derived fungus *Aspergillus* sp. SF-5044 by Sohn and Oh [1]. This compound features a distinctive furano-furanone bicyclic lactone scaffold, which is of particular interest in medicinal chemistry due to its structure and potential biological activity. A recent study has reported a moderate antimicrobial activity of protulactone A against selected plant pathogens [2]. However, PLA did not exhibit significant antibacterial or antifungal effects against human pathogens, even at concentrations as high as 2.5 mM [3]. To overcome these limitations, our research explores the development of hybrid compounds combining PLA with styryl lactones-molecules known for their notable antimicrobial properties. This approach aims to enhance the biological potential of PLA through rational design and molecular hybridization.



(+)-protulactone A

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## Geospatial Mapping of Antimicrobial Resistance in the Urban and Rural Aquatic Environments of Bangladesh

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The spatial distribution of clinically important antibiotic resistant bacteria (ARB) and associated genes is important to identify the environmental distribution of contamination and ‘hotspots’ of antimicrobial resistance (AMR). We conducted an integrated survey of AMR in drinking water, wastewater and surface water (rivers and ponds) in three settings in Bangladesh: rural households, rural poultry farms, and urban food markets. Spatial mapping was conducted via geographic information system (GIS) using ArcGIS software. Samples (n = 397) were analyzed for the presence of extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* (*ESBL-Ec*), carbapenem-resistant *E. coli* (*CR-Ec*) and resistance genes (*blaCTX-M-1*, *blaNDM-1*). In rural households, 5% of drinking water supply samples tested positive for *ESBL-Ec*, and a high proportion of wastewater, pond and river water samples were positive for *ESBL-Ec* (90%, 76%, and 85%, respectively). In poultry farms, 10% of drinking water samples tested positive for *ESBL-Ec* compared to a high prevalence in wastewater, pond and river water (90%, 68%, and 85%, respectively). *CR-Ec* prevalence in household wastewater and pond water was relatively low (8% and 5%, respectively) compared to river water (33%). In urban areas, 38% of drinking water samples and 98% of wastewater samples from food markets tested positive for *ESBL-Ec* while 30% of wastewater samples tested positive for *CR-Ec*. Wastewaters had the highest concentrations of *ESBL-Ec*, *CR-Ec*, *blaCTXM-1* and *blaNDM-1* and these were significantly higher in urban compared to rural samples ( $p < 0.05$ ). *ESBL-Ec* is ubiquitous in drinking water, wastewater and surface water bodies in both rural and urban areas of Bangladesh. *CR-Ec* is less widespread but found at a high prevalence in wastewater discharged from urban food markets and in rural river samples. Surveillance and monitoring of antibiotic-resistant organisms and genes in waterbodies is an important first step in addressing environmental dimensions of AMR.



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## Chemical Profiling of Virulence-related Enzymes

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Antibiotic resistance is a growing global health crisis, highlighting the need for novel therapeutic approaches that extend beyond conventional antibiotics. One promising direction focuses on **virulence-related bacterial enzymes**, such as those involved in quorum sensing and extracellular proteolysis, which play critical roles in bacterial adaptation and pathogenicity. Despite their importance, many of these enzymes remain poorly understood, restricting our capacity to develop targeted anti-virulence strategies.

In this presentation, I will introduce “**activity-based protein profiling**” (ABPP), a chemical biology technique that uses molecular probes to detect and characterize active enzymes in complex biological samples. First, I will demonstrate how ABPP provided insights into PqsD and HmqD, two enzymes essential for the production of quinolone quorum-sensing signals in *Pseudomonas aeruginosa* and *Burkholderia* species. By applying targeted “**activity-based probes**” (ABPs) in a live-cell screening strategy, we identified potent, biocompatible inhibitors that effectively suppressed the production of these quinolone signalling molecules in *P. aeruginosa* and *B. ambifaria* [1-3]

Building on these findings, my newly established independent research group now focuses on extracellular bacterial proteases as potential drug and diagnostic targets. Their extracellular location makes them particularly attractive for inhibitor design and biomarker discovery. I will present recent data on ABPs targeting **IgA1 proteases (IgA1Ps)** from *Haemophilus influenzae*, which cleave host IgA1 antibodies to evade immune responses. This is particularly relevant in chronic obstructive pulmonary disease (COPD), where *H. influenzae* is a major contributor to infection and disease exacerbation. By integrating substrate-mimicking recognition elements and serine-reactive electrophiles into our ABPs, we achieved selective labelling of *H. influenzae* IgA1P without cross-reactivity toward related bacterial or human proteases, even in complex samples.

These probes serve as powerful tools to study the roles of these enzymes during infection, providing critical insights into their contribution to disease. At the same time, they support the validation of these enzymes as drug targets and diagnostic markers, laying the groundwork for new anti-virulence strategies. Ongoing work in our lab is expanding ABP development to additional extracellular proteases implicated in bacterial pathogenesis, thereby unlocking new strategies to combat infectious diseases.

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## Antibiotic resistance in the neonatal intensive care unit and how much we know about it?

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Antibiotic resistance is a major public health threat. Many bacteria exhibit resistance to multiple antibiotics. Antimicrobial therapy is a common and daily practice in Neonatal Intensive Care Units and antibiotics are the most commonly used medications.

Gram-positive pathogens are more common causes of infections than gram-negative and mycotoxin-producing pathogens. Staphylococcal species, most commonly *S. epidermidis* and *S. aureus*, cause approximately 60-70% of infections. Gram-negative bacilli cause approximately 15-20% of infections, most commonly late-onset sepsis and also pneumonia associated with or after mechanical ventilation, and *Candida* spp. cause approximately 10% of infections in NICUs. The reason for the widespread use of antibiotics in NICU is closely related to the fact that newborns are a group at high risk for infections, especially those with risk factors such as premature birth and low birth weight, which increase the need for prolonged hospitalization and invasive procedures. Most immune mechanisms are not yet activated at birth, so neonates are immunodeficient compared to adults. Since a fetus's immunity develops during life in the womb, this immunodeficiency is even more severe in children born prematurely. As a result, survival, especially in premature infants, often depends on antibiotics.

Antibiotic resistance is a natural process that occurs over time due to genetic changes in pathogenic microbes. Both the frequency with which antibiotics are administered and the potential adverse effects of antibiotic administration differ between premature and full-term infants.

The main causes of antibiotic resistance remain poor infection control practices and misuse of antibiotics, including taking them unnecessarily, taking the wrong antibiotic, and taking them for an unnecessarily long time.

These organisms can be transmitted to infants if infection control practices are not followed, or they can be acquired through exposure to antibiotics.

Therefore, meticulous infection prevention—including hand hygiene, contact precautions, and selective decolonization—and antibiotic administration are important strategies to minimize drug resistance in RTIN.

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## Trends in Antibiotic Utilization and Antimicrobial Resistance in Albania (2011–2021)

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Antimicrobial resistance continues to pose a major global health threat, driven in part by the inappropriate use of high-resistance potential 'Watch' antibiotics. In Albania, data on antibiotic consumption patterns have been limited, particularly during the COVID-19 pandemic, when antibiotic use increased despite limited evidence of bacterial coinfections.

Between 2011 and 2019, overall antibiotic consumption in Albania declined from **27.4 to 18.8 defined daily doses (DDs) per 1,000 inhabitants per day**, a trend likely supported by an ageing population and improved healthcare infrastructure. Despite this overall reduction, the proportion of 'Watch' antibiotics among the ten most frequently used antibiotics rose markedly—from **10% in 2011 to 70% in 2019**.

This downward trend in overall antibiotic use reversed during the COVID-19 pandemic, with consumption rising to **25.1 DDs in 2021**. The use of 'Watch' antibiotics continued to grow, comprising **82% of the top 10 antibiotics** by 2021 (DD basis).

The increased reliance on 'Watch' antibiotics, especially during and after the pandemic, highlights the urgent need for strengthened antimicrobial stewardship initiatives and educational programs in Albania to promote responsible antibiotic use and curb the spread of AMR.

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